Biological Activity of a Phloroglucinol Glucoside Derivative from *Conyza aegyptiaca*

Ahmed A. Mahmoud[a], Shar S. Al-Shihry[a], and Mohamed-Elamir F. Hegazy[b,*]

[a] Chemistry Department, College of Science, King Faisal University, P. O. Box 1759, Hofuf 380, Saudi Arabia

[b] Chemistry of Medicinal Plants Department, National Research Centre, El-Tahrir St., Dokki, Giza, 1622, Egypt. Fax: 20-23 37 09 31. E-mail: elamir77@yahoo.com

* Author for correspondence and reprint requests

Z. Naturforsch. **64c**, 513–517 (2009); received April 15/May 4, 2009

The phloroglucinol glucoside derivative [2,4-dihydroxy-6-(β-D-glucopyranosyloxy)phenyl]-butan-1-one (**1**), roseoside (**2**), and kaempferol-3-*O*-β-D-glucopyranoside (**3**) were isolated from the aerial parts of *Conyza aegyptiaca* (L.). To the best of our knowledge, this is the first isolation of compounds **1**–**3** from *C. aegyptiaca*. Their structures were determined by spectroscopic techniques including, IR, HR-EIMS, and extensive 500 MHz 1D- and 2D-NMR analyses (^{1}H, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC experiments). The antioxidant activity of **1**, using the DPPH assay, was investigated; in addition, **1** was investigated against different types of cell lines, including Hep-G2, HCT-116, and RAW 264.7 for its cytotoxic effects. Also, this is the first report on the activity of **1**.

Key words: Conyza aegyptiaca, Asteraceae, Phloroglucinol Glucoside, Antioxidant

Introduction

The genus *Conyza* (L.) (Asteraceae) is an annual, biennial or perennial herbaceous plant. It consists of 80–100 species growing in tropical and subtropical areas of the world (Beentje, 2002; Chai *et al.*, 2008). *Conyza aegyptiaca* (L.) Aiton is an aromatic herb mainly distributed in Africa, tropical Asia and Australia (Chai *et al.*, 2008). The plant is used in folk medicine as an anthelmintic, a body-wash for convalescents, and a soothing for skin diseases (Burkill, 1985). Previous pharmacological studies have shown that its polar extracts possess antiviral and antimicrobial activities (Anani *et al.*, 2000). Phytochemical investigations on *C. aegyptiaca* have led to the isolation of diterpenes (Zdero *et al.*, 1990), triterpenes (Metwally, 1989; Hammouda *et al.*, 1978), sesquiterpenes (Zdero *et al.*, 1990; Metwally and Dawidar, 1984a, b), and flavonoids (El-Karemy *et al.*, 1986; Ismail *et al.*, 1979). In continuation of our phytochemical studies on the members of the family Asteraceae, we have reinvestigated the aerial parts of *C. aegyptiaca* collected from Egypt. The phloroglucinol glucoside derivative [2,4-dihydroxy-6-(β-D-glucopyranosyloxy)phenyl]-butan-1-one (**1**) (Jin *et al.*, 2008), roseoside (**2**) (Bhakuni *et al.*, 1974; Pabst *et al.*, 1992; Otsuka *et al.*, 1995), and kaempferol-3-*O*-β-D-glucopyranoside (**3**) (Park *et al.*, 1991) were isolated from the CH_2Cl_2/MeOH extract of this species. The structures of these compounds were determined by spectroscopic techniques, including HR-EIMS, and extensive 500 MHz 1D- and 2D-NMR analyses (^{1}H, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC experiments). Additionally, the antioxidant activity and cytotoxic effects against different cell lines of compound **1** were reported for the first time.

Results and Discussion

Chromatographic separation of the CH_2Cl_2/MeOH extract of the aerial parts of *C. aegyptiaca* yielded three compounds, **1**–**3**, isolated for the first time form this species.

Compound **1** was isolated as a pale yellow powder with $[\alpha]_D^{25}$ –59.3° (*c* 0.015, MeOH). Its IR spectrum showed absorption bands due to a hydroxy group (ν = 3273 cm^{-1}) and a conjugated carbonyl group (ν = 1630 cm^{-1}). The molecular formula was found to be $C_{16}H_{22}O_9$ by high-resolution EIMS, which showed a molecular ion at *m/z* 358. The molecular formula was confirmed by ^{13}C NMR and DEPT analyses.

The presence of a glucosyl moiety was evident from the typical ^{1}H NMR signals at δ_H 5.04 (1H, d, J = 8.5 Hz, H-1′), 3.55 (1H, t, J = 8.5 Hz, H-2′), 3.51 (1H, t, J = 8.5 Hz, H-3′), 3.42 (1H, t,

J = 8.5 Hz, H-4′), 3.46 (1H, m, H-5′), 3.91 (1H, dd, J = 12.0, 1.8 Hz, H-6′a) and 3.73 (1H, dd, J = 12.2, 5.6 Hz, H-6′b) (Table I). Furthermore, these seven sugar proton signals showed correlations in the HMQC spectrum with six carbon resonances at δ_C 101.6 (d, C-1′), 74.7 (d, C-2′), 78.2 (d, C-3′), 71.1 (d, C-4′), 78.6 (d, C-5′) and 62.4 (t, C-6′), respectively, which were identical to standard values for the glucosyl group. The large coupling constant of the anomeric proton (8.5 Hz) between H-1′ and H-2′ indicated β-configuration of the D-glucopyranoside unit.

The ^{13}C NMR spectrum of **1** showed, in addition to the signals resulting from the glucopyranoside moiety, ten signals corresponding to the aglycone part. These signals were assigned from the DEPT and HMQC analyses to one methyl group [δ_C 14.2 (C-10)], two aliphatic methylene groups [δ_C 47.2 (C-8) and 19.1 (C-9)], one conjugated carbonyl group [δ_C 207.5 (C-7)], and six aromatic carbon atoms, indicating the presence of an aromatic ring, four of them were substituted [δ_C 106.7 (C-1), 167.4 (C-2), 165.7 (C-4), 162.1 (C-6)] and two upfield unsubstituted [δ_C 98.3 (C-3), 95.3 (C-5)]. The three aromatic signals with chemical shifts of δ_C 162.1, 165.7 and 167.4 indicated the attachment to an electron-withdrawing group, for example a hydroxy group, as they were deshielded. These carbon resonances are typical for a phloroglucinol (1,3,5-trihydroxylated benzene) (Shiu and Gibbons, 2006; Drewes and Van Vuuren, 2008; Ali *et al.*, 2004; Van Klink *et al.*, 2005; Supudompol *et al.*, 2004).

The ^{1}H NMR spectrum confirmed the characteristic features of 1,2,4,6-tetrasubstituted benzene by a pair of *meta*-coupled aromatic protons [δ_H 5.95 (1H, d, J = 2.1 Hz, H-3) and 6.17 (1H, d, J = 2.1 Hz, H-5)], a methyl triplet [δ_H 0.96 (3H, t, J = 7.0 Hz, H-10)], and two aliphatic methylene groups, one of them most probable adjacent to a conjugated keto group [δ_H 3.09 and 3.11 (m, H-8a and H-8)] and the other one was shielded [δ_H 1.68 (2H, m, H-9)].

All these protons constituted the aglycone part of the molecule. The assignments of all the protons and their connectivities to adjacent protons and carbon atoms could be substantiated from the results of the 2D ^{1}H-^{1}H COSY and HMQC spectra. The presence of a propyl group was traced from the C-10 methyl protons (δ_H 0.96), which exhibited ^{1}H-^{1}H COSY correlations with the C-9 methylene protons (δ_H 1.68), and these further exhibited vicinal coupling with the C-8 methylene protons (δ_H 3.09 and 3.11). Furthermore, these propyl pro-

Table I. ^{1}H, ^{13}C NMR, and HMBC spectral data of **1** ($CDCl_3$, 500 MHz, δ in ppm).

Position	δ_C	DEPT	δ_H (mult.)	HMBC
Aglycone				
1	106.7	C	–	
2	167.4	C	–	
3	98.3	CH	5.95 (1H, d, J = 2.1 Hz)	C-1, C-2, C-4, C-5
4	165.7	C	–	
5	95.3	CH	6.17 (1H, d, J = 2.1 Hz)	C-1, C-3, C-4, C-6
6	162.1	C	–	
7	207.5	C		
8	47.2	CH_2	3.11 (1H, m)	C-7, C-9, C-10
8a			3.09 (1H, m)	C-7, C-9, C-10
9	19.1	CH_2	1.68 (2H, m)	C-7, C-8, C-10
10	14.2	CH_3	0.96 (3H, t, J = 7.0 Hz)	C-8, C-9
Glucosyl				
1′	101.6	CH	5.04 (1H, d, J = 8.5 Hz)	C-6, C-3′
2′	74.7	CH	3.55 (1H, t, J = 8.5 Hz)	
3′	78.2	CH	3.51 (1H, t, J = 8.5 Hz)	
4′	71.1	CH	3.42 (1H, t, J = 8.9 Hz)	
5′	78.6	CH	3.46 (1H, m)	
6′a	62.4	CH_2	3.91 (1H, dd, J = 12.0, 1.8 Hz)	
6′b			3.73 (1H, dd, J = 12.2, 5.6 Hz)	

Assignments by ^{1}H-^{1}H COSY, HMQC and HMBC experiments. Carbon multiplicities were determined by DEPT experiments.

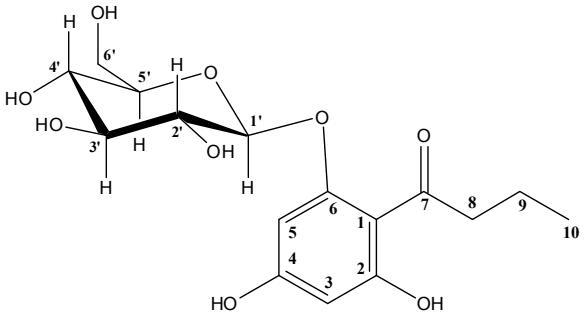

Fig. 1. Chemical structure of [2,4-dihydroxy-6-(*β*-D-glucopyranosyloxy)phenyl]-butan-1-one (**1**).

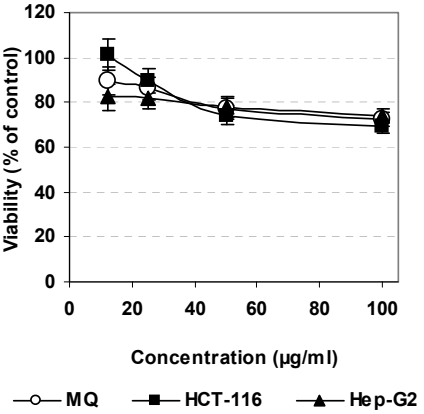

Fig. 2. The cytotoxicity of different doses of the extract on hepatocellular carcinoma Hep-G2 cells (triangled line), colon carcinoma HCT-116 cells (squared line), and Raw 264.7 macrophages (MQ, circled line), as measured by the MTT assay. The results are presented as the percentage of control cells (mean ± SD, $n = 4$).

tons showed correlations in the HMQC spectrum with three carbon signals at δ_C 14.2 (CH_3, C-10), 19.1 (CH_2, C-9) and 47.2 (CH_2, C-8), respectively, which indicated together with a keto group at δ_C 207.5 that a butanoyl group was present.

Based on all these data, **1** was assumed to be a butanoyl derivative of phloroglucinol glucoside. The complete structure of **1** and the location of the butanoyl group and the glucosyl moiety at the aromatic ring were confirmed by the chemical shifts and HMBC experiments. The location of the butanoyl group at C-1 was established from the ^{13}C-chemical shift of C-1 (δ_C 106.7), which was similar to those reported for phloroglucinols substituted with similar acyl groups (Shiu and Gibbons, 2006; Drewes and Van Vuuren, 2008; Ali *et al.*, 2004; Van Klink *et al.*, 2005; Supudompol *et al.*, 2004). Furthermore, the HMBC correlation between the anomeric sugar proton H-1′ at δ_H 5.04 and the oxygenated aromatic carbon C-6 at δ_C 162.1 established the presence of the glucopyranosyloxy group at C-6.

Therefore, **1** was identified as [2,4-dihydroxy-6-(*β*-D-glucopyranosyloxy)phenyl]-butan-1-one (recorded in $CDCl_3$) (Fig. 1). Compound **1** was recently isolated form *Solidago altissima* (Jin *et al.*, 2008) and its data was recorded in CD_3OD.

Compound **2**, was isolated as a pale yellow powder $[\alpha]_D$ +20.3º (*c* 0.001, MeOH). The structure was established based on ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC, EIMS and HR-EIMS data. Its IR spectrum showed absorption bands due to a hydroxy group (ν = 3250 cm^{-1}) and a conjugated carbonyl group (ν = 1650 cm^{-1}). The molecular formula was found to be $C_{19}H_{30}O_8$ by high-resolution EIMS, which showed a molecular ion at *m/z* 386.1941. This formula was confirmed by ^{13}C NMR and DEPT analyses. The complete 1D and 2D spectroscopic analyses established that compound **2** is roseoside (Bhakuni *et al.*, 1974; Pabst *et al.*, 1992; Otsuka *et al.*, 1995), which was isolated for the first time from *C. aegyptiaca*.

Compound **3** was identical in ^{1}H NMR and ^{13}C NMR spectra to kaempferol-3-*O*-*β*-D-glucopyranoside (Park *et al.*, 1991).

For the investigation of the cytotoxic effect of **1** against different cell lines (Hep-G2, HCT-116 and MQ cells), cells were treated with and without different doses of the extract for 24 h, and submitted to the MTT assay, a metabolic cytotoxicity assay. The experiment showed that the extract has no significant cytotoxic effect ($P > 0.05$) on all types of cell lines, as shown in Fig. 2.

To investigate the radical scavenging activity we submitted the purified compound **1** to the DPPH assay, which revealed that **1** possesses a moderate scavenging activity against DPPH radicals with an SC50 value of 25.4 *μ*g/mL, compared with the scavenging activity of the known antioxidant ascorbic acid (SC50 8.6 *μ*g/mL).

Experimental

General

^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$) and 2D spectra (^{1}H-^{1}H COSY, HMQC, HMBC) were recorded on a JEOL LA500 MHz

spectrometer with TMS as an internal standard. The IR spectrum (KBr) was taken on a HORIBA FT-720 spectrometer. Optical rotation was determined with a HORIBA SEPA-300 spectropolarimeter. MALDI-TOF mass spectra were recorded on an Applied Biosystems Voyager-DE PRO mass spectrometer. Column chromatography was carried out on silica gel 60 (Merck; 230–400 mesh) and Sephadex LH-20 (Pharmacia Co., Tokyo, Japan). TLC: precoated silica gel $60F_{254}$ plates (Merck); preparative TLC: silica gel PF_{254} (Merck, 200 × 200 × 0.25 mm).

Plant material

Aerial parts of *C. aegyptiaca* (L.) were collected from South Sinai, Egypt in March 2002. A voucher specimen of the collection was identified by Prof. Dr. Mohei Kamel and was deposited at the Department of Botany, El-Minia University, Egypt.

Extraction and isolation

The air-dried aerial parts (1 kg) were powdered and extracted with CH_2Cl_2/MeOH (1:1) (10 L) at room temperature. The extract was concentrated *in vacuo* to obtain a residue of 60 g. The residue was prefractionated by CC (6 × 100 cm) on silica gel (700 g) eluted with *n*-hexane followed by CH_2Cl_2 and finally CH_2Cl_2/MeOH (85:15) (3 L each of the solvents). The 15% MeOH fraction was subjected to silica gel column chromatography (3 × 60 cm), eluted with *n*-hexane/CH_2Cl_2/MeOH (7:4:1.5), to afford a mixture of **1** and **2**. Further purification on a Sephadex LH-20 column, eluted with CH_2Cl_2/MeOH (2:1), and preparative TLC eluted with CH_2Cl_2/MeOH (7:1) afforded **1** (30 mg) and **2** (12 mg) in a pure form.

Cell culture

Several human cell lines were used to test the anticancer activity, including hepatocellular carcinoma (Hep-G2) and colon carcinoma (HCT-116) cells, and Raw murine macrophages (RAW 264.7; ATCC, VA, USA). Cells were routinely cultured in DMEM (Dulbecco's modified Eagle's medium), except for the RAW 264.7 cells, which were grown in RPMI-1640 medium at 37 ºC in humidified air containing 5% CO_2. Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/mL penicillin G sodium, 100 units/mL streptomycin sulfate, and 250 ng/mL amphotericin B. Monolayer cells were harvested by trypsin/EDTA treatment, except for RAW 264.7 cells, which were collected by gentle scraping. The tested extract was dissolved in dimethyl sulfoxide (DMSO, 99.9%, HPLC grade) and diluted 1000-fold in the assays. In the cellular experiments, results were compared with DMSO-treated cells. Extract dilutions were tested before the assays for endotoxin using the Pyrogent® Ultra gel clot assay (Sigma Chemical Co., USA); they were found to be endotoxin-free. All experiments were repeated four times and the data was presented as mean ± SD. All culture material was obtained from Cambrex BioScience (Copenhagen, Denmark), and all chemicals were from Sigma (USA).

Cytotoxicity assay

The antiproliferative activity of the extract against hepatocellular carcinoma (Hep-G2) and colon carcinoma (HCT-116) cells and Raw 264.7 macrophages (MQ) was estimated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay (Hansen *et al.*, 1989). The yellow tetrazolium salt of MTT is reduced by mitochondrial dehydrogenases in metabolically active cells to form insoluble purple formazan crystals, which are solubilized by the addition of a detergent. Cells ($5 \cdot 10^4$ cells/well) were incubated with various concentrations of the compounds at 37 ºC in FBS-free medium, before submitted to the MTT assay. The absorbance was measured with an ELISA reader (Bio-Rad, München, Germany) at 570 nm. The relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt. The data is expressed as the mean percentage of viable cells as compared to the respective control cultures treated with DMSO.

Antioxidant assay

The antioxidant capacity of the tested compound was studied through its scavenging activity against 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals (Gerhäuser *et al.*, 2003; Van Amsterdam *et al.*, 1992). The bleaching of DPPH was monitored at an absorbance of 515 nm. The percentage of DPPH bleaching utilized for SC50 (half maximal scavenging concentration) was calculated as

follows: 0% is the absorbance of DPPH with solvents (ethanol) and 100% is the absorbance of DPPH with an efficient scavenger (10 mM ascorbic acid, AA).

Statistical analysis

MTT assay data were analyzed using two-factorial analysis of variance (ANOVA), including first-order interactions (two-way ANOVA), followed by the Tukey post hoc test for multiple comparisons. $P < 0.05$ indicated statistical significance.

[2,4-Dihydroxy-6-(β-D-glucopyranosyloxy)phenyl]-butan-1-one (**1**): Pale yellow powder, $[\alpha]_D^{25}$ −59.3° (*c* 0.015, MeOH). – IR (KBr): ν_{max} = 3273, 1630, 1585, 1460, 1075 cm^{-1}. – HR-EIMS: 358.1245 (calcd. for $C_{16}H_{22}O_9$ 358.1264). – EIMS: *m/z* (rel. int.) = 358 $[M]^+$ (70), 330 (15). – ^1H, ^{13}C NMR and HMBC analyses: see Table I.

Acknowledgements

The authors acknowledge the Deanship Scientific Research at King Faisal University for the financial support of this work under research program No. 8069 (2007).

Ali Z., Ito T., Tanaka T., Nakaya K., Murata J., and Iinuma M. (2004), Acetophenone *C*-glucosides and stilbene *O*-glucosides in *Upuna borneensis*. Phytochemistry **65**, 2141–2146.

Anani K., Hudson J. B., De Souza C., Akpagana K., Tower G. H. N., Arnason J. T., and Gbeassor M. (2000), Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharm. Biol. **38**, 40–45.

Beentje H. J. (2002), Flora of Tropical East Africa, Vol. 1. CRC Press, London, p. 315.

Bhakuni D. S., Joshi P. P., Uprety H., and Kapil R. S. (1974), Roseoside, a C_{13} glycoside from *Vinca rosea*. Phytochemistry **13**, 2541–2543.

Burkill H. M. (1985), The Useful Plants of West Tropical Africa, Vol. 1. Royal Botanica Gardens, Kew.

Chai X., Su Y.-F., Gua L.-P., Wu D., Zhang J.-F., Si C.-L., Kim J.-K., and Bae Y.-S. (2008), Phenolic constituents from *Conyza sumatrensis*. Biochem. Syst. Ecol. **36**, 216–218.

Drewes S. E. and Van Vuuren S. F. (2008), Antimicrobial acylphloroglucinols and dibenzyloxy flavonoids from flowers of *Helichrysum gymnocomum*. Phytochemistry **69**, 1745–1749.

El-Karemy Z. A. R., Mansour R. M. A., Fayed A. A., and Saleh N. A. M. (1986), The flavonoids of local members of Asteraceae (Compositae). Biochem. Syst. Ecol. **15**, 53–55.

Gerhäuser C., Klimo K., Heiss E., Neumann I., Gamal-Eldeen A., Knauft J., Liu J. U., Sitthimonchai S., and Frank N. (2003), Mechanism-based *in vitro* screening of potential cancer chemopreventive agents. Mutat. Res. **523–524**, 163–172.

Hammouda F. M., Rizk A. M., Seif El-Nasr M. M., and El-Kady S. E. M. (1978), Phytochemical investigation of *Conyza dioscoridis* and *C. aegyptiaca*. Fitoterapia **49**, 51–52.

Hansen M. B., Nielsen S. E., and Berg K. (1989), Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods **119**, 203–210.

Ismail S. I., Hammouda F. M., Rizk A. M., and El-kady S. (1979), Flavonoid constituents of *Conyza dioscoridis* L. and *Conyza aegyptiaca* Ait. Pharmazie **34**, 112.

Jin H., Ogino K., Fujioka T., Yoshida M., and Ishimaru K. (2008), A new acylphloroglucinol glycoside from *Solidago altissima* L. J. Nat. Med. **62**, 199–201.

Metwally M. A. (1989), New triterpene from *Conyza aegyptiaca* L. Z. Naturforsch. **44b**, 1584.

Metwally M. A. and Dawidar A. A. M. (1984a), Constituents of *Conyza aegyptiaca* L. Pakistan J. Sci. Ind. Res. **27**, 213–214.

Metwally M. A. and Dawidar A. A. M (1984b), Constituents of *Conyza aegyptiaca* L. Pharmazie **38**, 575–576.

Otsuka H., Yao M., Kamada K., and Takeda Y. (1995), Alangionosides G–M: glycosides of megastigmane derivatives from the leaves of *Alangium premnifolium*. Chem. Pharm. Bull. **34**, 754–759.

Pabst A., Barron D., Semon E., and Schreier P. (1992), Two diastereomeric 3-oxo-α-ionol β-D-glucosides from raspberry fruit. Phytochemistry **31**, 1649–1652.

Park H. J., Young H. S., Park K. Y., Rhee S. H., Chung H. Y., and Choi J. S. (1991), Flavonoids from the whole plants of *Orostachys japonicus*. Arch. Pharm. Res. **14**, 167–171.

Shiu W. K. P. and Gibbons S. (2006), Anti-staphylococcal acylphloroglucinol from *Hypericum beanii*. Phytochemistry **67**, 2568–2572.

Supudompol B., Likhitwitayawuid K., and Houghton P. J. (2004), Phloroglucinol derivatives from *Mallotus pallidus*. Phytochemistry **65**, 2589–2594.

Van Amsterdam F. T. M., Roveri A., Maiorino M., Ratti E., and Ursini F. (1992), Lacidipine: a dihydropyridine calcium antagonist with antioxidant activity. Free Rad. Biol. Med. **12**, 183–187.

Van Klink J. W., Larsen L., Perry N. B., Weavers R. T., Cook G. M., Bremer P. J., MacKenzie A. D., and Kirikae T. (2005), Triketones active against antibiotic-resistant bacteria: Synthesis, structure-activity relationships, and mode of action. Bioorg. Med. Chem. **13**, 6651–6662.

Zdero C., Ahmed A. A., Bohlmann F., and Mungai G. M. (1990), Diterpenes and sesquiterpene xylosides from East Africa *Conyza* species. Phytochemistry **29**, 3167–3172.