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Phytochemistry and antimycobacterial activity of *Chlorophytum inornatum*

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Abstract

In a project to investigate plant derived natural products from the Liliaceae with activity against fast-growing strains of mycobacteria, we have identified two new metabolites from *Chlorophytum inornatum*. The active principle, a new homoisoflavanone (**1**) was identified as 3-(4′-methoxybenzyl)-7,8-methylenedioxy-chroman-4-one. The metabolite assigned as 7-(1′-hydroxyethyl)-2-(2″-hydroxyethyl)-3,4-dihydrobenzopyran (**2**) was characterised by extensive 1- and 2D NMR spectroscopy. The antimycobacterial activity of this plant was mainly due to the homoisoflavonoid which exhibited minimum inhibitory values ranging from 16–256 μg/ml against four strains of fast-growing mycobacteria.

Keywords: *Chlorophytum inornatum*; Homoisoflavanone; Liliaceae; *Mycobacterium*; Antibacterial; Mycobacteria

1. Introduction

At present there is a real need for new classes of antibacterials to deal with emerging resistant strains, particularly in the genera *Mycobacterium* and *Staphylococcus*. The genus *Mycobacterium* is responsible for tuberculosis (TB) and other infections caused by fast-growing mycobacteria (FGM) such as *M. abscessus* (Scholze et al., 2005). These FGM species are notoriously difficult to treat and cause infections in children with cystic fibrosis (Sermet-Gaudelus et al., 2003). Multidrug-resistance in some species of FGM (Sander et al., 2000) and TB causing species (Colangeli et al., 2005) has been encountered and it is likely that these mechanisms will be more clinically relevant in the future. There is therefore a requirement for new classes of antibacterials which have activity against these strains. In a project to meet these needs, we have been screening plants of the Liliales, particularly of the Alliaceae and Liliaceae families. These groups produce bulbs as part of their reproductive system and our rationale is that such bulbs are in contact with actinomycetes in the soil and will have evolved an antimicrobial defence against these filamentous bacteria. Members of the actinomycete genus *Mycobacterium* are commonly present in soil and it is possible that such an antimicrobial defence may be useful to find leads against species of this group. Allicin, the major antimicrobial principle of the garlic group (*Allium*), has been extensively studied for its antibacterial properties some considerable time ago (Cavallito et al., 1944; Cavallito and Bailey, 1944; Cavallito et al., 1945). Garlic has also been used clinically to treat patients with TB in the early part of the 20th Century (Bolton et al., 1982) and is popularly known as 'Russian penicillin'.

These findings prompted us to conduct an evaluation of other less well chemically and biologically characterised members of the Liliaceae and Alliaceae groups and a collection of the subterranean parts of the poorly studied species *Chlorophytum inornatum* Ker Gawl. (Liliaceae) was made in Ghana. There is little phytochemical data available on this genus although *C. comosum*, a popular houseplant known as the spider plant or grass lily, along with

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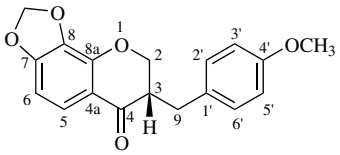
other members of the genus, is of ethnobotanical use in areas of Africa and India (Tabuti et al., 2003 and Tandon et al., 1992). In southern Chinese folk medicine *C. comosum* is also used in the treatment of bronchitis, fractures and burns (Mimaki et al., 1996).

Here we report on the bioassay-guided fractionation of extracts of *C. inornatum* against fast-growing strains and on new phytochemistry of this species.

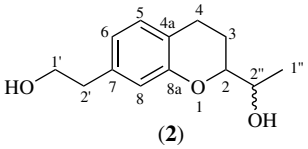
2. Results and discussion

Antibacterial activity was concentrated in the hexane extract (512 μg/ml) and bioassay-guided fractionation led to the isolation of compound **1** as a white solid. The molecular formula of compound **1** was assigned as $C_{18}H_{16}O_5$ on the basis of high-resolution ESI-MS $[M+H]^+$ (313.1085). The ^1H NMR spectrum (Table 1) displayed a chemical shift pattern indicative of a 3-benzyl-4-chromanone type homoisoflavanone skeleton previously isolated from the Liliaceae and Hyacinthaceae (Anh et al., 2003; Adinolfi et al., 1984). Analysis of the 1D and 2D NMR spectra revealed the presence of methylenedioxy and methoxyl substituents on the aromatic A and B rings, respectively. Two doublets (δ 6.87 and 7.17, $J = 8.5$ Hz) integrating for two protons each were typical for an aromatic AA′BB′ system for ring B, with a strong HMBC signal between the *para* substituted methoxyl (δ 3.81) and C-4′ (δ 158.4). Correlations observed in the NOESY spectrum from the methoxyl to the aromatic protons H-3′ and H-5′ (δ 6.87) further confirmed this arrangement. Two coupled proton doublets of ring A (δ 7.61 and 6.62) showed an *ortho* coupling ($J = 8.0$ Hz), while the HMBC correlation between the proton at δ 7.61 and the C-4 carbonyl (δ 192.1) identified the protons as H-5 and H-6, respectively. The difference between the ^1H chemical values for H-5 and H-6 can be attributed to the observations that H-5 is β to the C-4 carbonyl and that there is likely to be a *peri* through space deshielding interaction between H-5 and this carbonyl moiety.

The methylenedioxy group was positioned at C-7 and C-8 by HMBC correlations for the methylenedioxy protons to the aromatic quaternary carbons C-7 and C-8. This positioning was further confirmed by HMBC correlations from H-5 to the carbonyl at C-4 and the quaternary carbon C-4a. Furthermore, H-6 displayed HMBC correlations to C-7 and C-8. The COSY spectrum showed a $–CH_2–CH–CH_2–$ system reminiscent of a homoisoflavanone with C-2, C-3 and C-9 assigned through comparison with the literature (Adinolfi et al., 1984) and confirmed by 2D NMR experiments. This system was accordingly linked to the B ring through HMBC correlations from H_2-9 to the aromatic carbons C-2′ and C-6′. The compound was therefore determined to be 3-(4′-methoxybenzyl)-7,8-methylenedioxychroman-4-one, and is described here for the first time. Measurement of the specific optical rotation of **1** gave a result of +18.9°. Comparison with the results for homoisoflavanones with an *R* configuration described in Amschler et al. (1996) ([α] = −46.2 and −38.0) implied an *S* configuration for compound **1** (see Fig. 1).



(**1**)



(**2**)

Fractionation of the chloroform extract led to the isolation of a new metabolite, compound **2**. HRESI-MS of compound **2** suggested a molecular formula of $C_{13}H_{18}O_3$ $[M-H]^+$ (221.1168). The ^1H NMR spectrum (Table 2)

Table 1
^1H (500 MHz) and ^{13}C NMR (500 MHz) spectral data and ^1H–^{13}C long-range correlations of **1** recorded in $CDCl_3$

Position	^1H	^{13}C	^2J	^3J	^4J
2a	4.42 *dd* (11.5, 4.0)	70.0		C-4, C-9	C-4a
2b	4.26 *dd* (11.5, 7.0)		C-3	C-4, C-9	C-4a
3	2.86 *m*	48.1	C-9		
4	–	192.1			
4a	–	145.5			
5	7.61 *d* (8.0)	123.1	C-4a	C-4, C-7	
6	6.62 *d* (8.0)	103.5	C-7	C-8	C-8a
7	–	154.0			
8	–	134.4			
8a	–	117.3			
9a	2.72 *dd* (14.0, 10.5)	31.8	C-3	C-2, C-4, C-2′, C-6′	
9b	3.19 *dd* (14.0, 4.5)		C-3	C-2, C-4, C-2′, C-6′	
1′	–	130.0			
2′/6′	7.17 *d* (8.5)	130.1	C-1′, C-3′	C-9, C-4′, C-6′	C-5′
3′/5′	6.87 *d* (8.5)	114.1	C-2′, C-4′	C-1′, C-5′	C-6′
4′	–	158.4			
O–CH_2–O	6.09 *d* (1.0)	102.7		C-7, C-8	
OMe (4′)	3.81 *s*	55.3		C-4′	

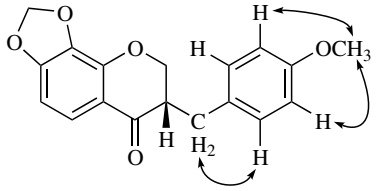


Fig. 1. Key NOESY correlations for compound **1**.

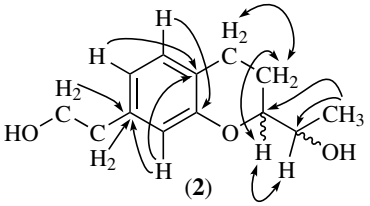


Fig. 2. Key HMBC (single headed arrows) and COSY (double headed arrows) correlations for compound **2**.

revealed three aromatic resonances at δ 6.67 (*d*, $J_{ortho} = 8.0$ Hz, H-5), δ 6.65 (*d*, $J_{meta} = 2.0$ Hz, H-8) and δ 6.52 (*dd*, $J = 2.0$, 8.0 Hz, H-6) reminiscent of an ABD trisubstituted ring system. Further resonances included two oxygenated methine multiplets (δ 4.40 and 3.75), an oxygenated methylene triplet (δ 3.67, $J = 7.5$ Hz), two benzylic methylene triplets (δ 2.66, $J = 7.0$ Hz and 2.55, $J = 8.5$ Hz), two one-proton multiplets corresponding to a further methylene by HMQC analysis (δ 2.25 and 2.07) and a methyl doublet (δ 1.21, $J = 6.5$ Hz) completing the ^{1}H signals for compound **2**. HMBC correlations from the protons of the oxygenated methylene (δ 3.67, H-1′) to a methylene carbon at δ 39.7 (C-2′) and a quaternary aromatic carbon at δ 131.8 were suggestive of an hydroxyethyl substituent attached to an aromatic ring. This was further confirmed by a NOESY correlation between the methylene protons (CH_2-2′) and the aromatic protons (H-6 and H-8). This was supported by HMBC correlations between the protons of this methylene and C-7 (^2J) and C-6 and C-8 (^3J). H-5 gave a strong HMBC correlation (Fig. 2) to C-8a which was a deshielded aromatic quaternary carbon with a resonance reminiscent of an oxygen bearing carbon (δ 146.1).

The remaining resonances could be linked by analysis of the COSY spectrum (Fig. 2). A methyl doublet (H-1″, δ 1.21) displayed a single correlation to the first of the oxygenated methines at δ 3.75 (H-2″), which in turn coupled to the second oxygenated methine at δ 4.40 (H-2). H-2 subsequently coupled to a methylene (H_2-3, δ 2.25 and 2.07), which showed a final coupling to a deshielded methylene triplet of H-4 (δ 2.55), whose resonance was typical for a benzylic methylene and similar to that of CH_2-2′. This methylene must be attached at C-4a, *ortho* to C-5 as this is the only remaining free position on the aromatic core. The data were similar to other natural product benzopyran derivatives (Seeram et al., 1998) and we propose that a link between the oxygen at C-8a (neighbouring C-8) and C-2 is formed to give a substituted benzopyran derivative. From HRESI-MS this would mean that hydroxyl groups must be placed at C-1′ and C-2″. Theoretical ^{1}H NMR resonances were calculated using ChemDraw Ultra and are given in Table 2. These resonances show close correlation with experimental data, particularly for H-4, H-1′, H-2′, H-1″ and H-2″ and even the aromatic resonances have the same trend in magnitude. The main discrepancy is for H-2 (4.40 exp, 3.76 theoret.) and we propose that this is due to the deshielding effect of the hydroxyl at C-2″, resulting in a more deshielded signal for H-2. Compound **2** is therefore assigned as 7-(1′-hydroxyethyl)-2-(2″-hydroxyethyl)-3,4-dihydrobenzopyran and its NMR data are described here for the first time.

Table 2
^{1}H (500 MHz) and ^{13}C NMR (500 MHz) spectral data and ^{1}H–^{13}C long-range correlations for **2**, recorded in MeOD

Position	^{1}H	^{13}C	^2J	^3J	^{1}H[a]
2	4.40 *m*	85.9	–	–	3.76
3	2.25 *m*, 2.07 *m*	25.0	C-4	–	2.00
4	2.55 *t* (8.5)	29.5	C-3	–	2.55
4a	–	144.9	–	–	–
5	6.67 *d* (8.0)	116.3	C-7	C-8a, C-4a	6.96
6	6.52 *dd* (2.0, 8.0)	121.2	C-5, C-7	–	6.63
7	–	131.8	–	–	–
8	6.65 *d* (2.0)	117.1	C-7	C-4a, C-6	6.58
8a	–	146.1	–	–	–
1′	3.67 *t* (7.0)	64.6	C-2′	C-7	3.86
2′	2.66 *t* (7.0)	39.7	C-1′, C-7	C-6, C-8	2.74
1″	1.21 *d* (6.5)	19.0	C-2″	C-2	1.21
2″	3.75 *m*	70.2	–	–	3.89

[a] Calculated using Chemdraw Ultra 7.0.

Further fractionation of the hexane extract led to the isolation stigmasterol-3-*O*-glycoside-6′-palmitate (Lavaud et al., 1994), a racemic mixture of [25*R*]-5α-spirostane-2α,3β-diol (gitogenin) and [25*S*]-5α-spirostane-2α,3β-diol (neogitogenin) (Mimaki et al., 1996) and 4-hydroxy-3-methoxy-benzaldehyde (vanillin). Further fractionation of the chloroform extract yielded *trans*-*N*-(4-hydroxyphenethyl)-feruloylamide (Muñoz et al., 1996). The structures were elucidated from 1D and 2D NMR experiments and compared against chemical shift resonances in the literature. The structure of stigmasterol-3-*O*-glycoside-6′-palmitate was further confirmed by hydrolysis and GC-MS of the derivatised hydrolysate.

Compound **1** exhibited MIC values of 16–256 μg/ml against a range of fast-growing *Mycobacterium* species, comparing favourably with the control antibiotics ethambutol and isoniazid against *M. phlei* and *M. aurum* (Table 3). This is the first report of the antimycobacterial activity of a homoisoflavanone. The simple structure makes this compound an attractive target for synthesis and derivatisation to optimise the antibacterial activity. Compound **2** displayed moderate activity against *M. aurum* (64 μg/ml), however showed no inhibition against any of the other fast growing mycobacterial strains tested (Table 3).

Table 3
MICs of **1** and standard antibiotics in μg/ml

Strain	1	2	Ethambutol	Isoniazid
M. fortuitum	128	–	2	0.25
M. smegmatis	256	–	32	0.25
M. phlei	16	–	2	128
M. aurum	32	64	16	>256

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer. Chemical shift values (δ) are reported in parts per million (ppm) relative to appropriate internal solvent standard and coupling constants (J values) are given in Hertz. Mass spectra were recorded on a Finnigan MAT 95 high resolution, double focusing, magnetic sector mass spectrometer. Accurate mass measurement was achieved using voltage scanning of the accelerating voltage. This was nominally 5 kV and an internal reference of heptacosa was used. Resolution was set between 5000 and 10,000.

IR spectra were recorded on a Nicolet 360 FT-IR spectrophotometer and UV spectra on a Thermo Electron Corporation Helios spectrophotometer. Determination of specific optical rotation [α] was carried out using an ADP 200 Polarimeter, Bellingham and Stanley, with a 1 ml sample tube.

3.2. Plant material

The dried root material of *C. inornatum* was collected in Aburi, Ghana, in August 2003 and a herbarium specimen (ACH/114866J) was deposited at the Centre for Pharmacognosy and Phytotherapy.

3.3. Extraction and isolation

234.2 g of air-dried and powdered subterranean parts were extracted in a Soxhlet apparatus using sequential extraction by hexane (3 L), chloroform (3 L) and finally methanol (3 L). The hexane extract (5.68 g) was subjected to vacuum liquid chromatography (VLC) on silica gel (15 g) eluting with hexane containing 10% increments of ethyl acetate to yield 12 fractions. The fraction eluted in 60% hexane underwent further separation on reversed-phase SPE (Phenomenex Strata C18-E, 10 g/60 ml giga tubes) eluting with 100% MeOH. SPE fraction 1 exhibited an MIC of 16 μg/ml against *M. fortuitum* and was further fractionated by Sephadex (LH-20) and a final PTLC (Merck RP-18, 85% MeOH: 15% water) purification to yield compound **1** (5.3 mg).

The chloroform extract (8.78 g) underwent initial fractionation with Biotage flash chromatography on silica gel, eluting with hexane containing 10% increments of ethyl acetate to yield 12 fractions. The fraction eluted in 10% hexane was further separated by reversed-phase SPE eluting with 60% MeOH: 40% water. SPE fraction 1 was subsequently fractionated by PTLC (50% MeOH: 50% water) to yield compound **2** (1.6 mg).

3.4. Antibacterial assay

Mycobacterium species were acquired from the NCTC. Strains were grown on Columbia Blood agar (Oxoid) supplemented with 7% defibrinated Horse blood (Oxoid) and incubated for 72 h at 37 °C prior to minimum inhibitory concentration (MIC) determination. Bacterial inocula equivalent to the 0.5 McFarland turbidity standard were prepared in normal saline and diluted to give a final inoculum density of 5×10^5 cfu/ml. The inoculum (125 μL) was added to all wells and the microtitre plate was incubated at 37 °C for 72 h for *M. fortuitum*, *M. smegmatis* and *M. phlei*. For *M. aurum* the plate was incubated for 120 h. The MIC was recorded as the lowest concentration at which no bacterial growth was observed as previously described (Gibbons and Udo, 2000).

Ethambutol and isoniazid were used as positive controls.

3.5. 3-(4′-methoxybenzyl)-7,8-methylenedioxy-chroman-4-one (*1*)

Amorphous white solid; $[\alpha]^{21}D$ +18.9° (*c* 0.05, $CHCl_3$) λ_{max} (log ε): 242.5 (3.60), 290.0 (3.61) nm; IR ν_{max} (thin film) cm^{-1}: 3855.34, 3735.96, 2918.31, 2850.23, 2359.02, 1684.84, 1630.82, 1512.72, 1364.37, 1289.81, 1247.99, 1083.89, 1035.90, 773.15; ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 1; HR-MS (*m*/*z*): 313.1085 $[M + H]^+$ (calc. for $C_{18}H_{17}O_5$, 313.1071).

3.6. 7-(1′-hydroxyethyl)-2-(2″-hydroxyethyl)-3,4-dihydrobenzopyran (*2*)

Amorphous solid; $[\alpha]^{21}D$ +157.14° (*c* +0.11, MeOH) λ_{max}: 201.5, 280 (br band) nm; IR ν_{max} (thin film) cm^{-1}: 3344.18, 2930.08, 1570.34, 1516.19, 1423.57, 1270.75, 1219.38, 1129.49, 1051.84, 773.07, 663.16; ^1H NMR and ^{13}C NMR (MeOD): see Table 2; HR-MS (*m*/*z*): 221.1168 $[M - H]^+$ (calc. for $C_{13}H_{17}O_3$, 221.1177).

Acknowledgements

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References

Adinolfi, M., Barone, G., Belardini, M., Lanzetta, R., Laonigro, G., Parrilli, M., 1984. 3-Benzyl-4-chromanones from *Muscari comosum*. Phytochemistry 23 (9), 2091–2093.

Amschler, G., Frahm, A.W., Hatzelmann, A., Kilian, U., Müller-Doblies, D., Müller-Dolbies, U., 1996. Constituents of *Veltheimia viridifolia*; I. Homoisoflavanones of the bulbs. Planta Medica 62, 534–539.

Anh, N.T.H., Sung, T.V., Porzel, A., Franke, K., Wessjohann, L.A., 2003. Homoisoflavonoids from *Ophiopogon japonicus* Ker-Gawler. Phytochemistry 62, 1153–1158.

Bolton, S.G., Null, G., Troetel, W.M., 1982. The medical uses of garlic – fact and fiction. J. Am. Pharm. Assoc. 22, 40–43.

Cavallito, C.J., Bailey, J.H., 1944. Allicin, the antibacterial principle of *Allium sativum*. II. Determination of the chemical structure. J. Am. Chem. Soc. 66, 1952–1954.

Cavallito, C.J., Buck, J.S., Suter, C.M., 1944. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. 66, 1950–1951.

Cavallito, C.J., Bailey, J.H., Buck, J.S., 1945. The antibacterial principle of *Allium sativum*. III. Its precursor and essential oil of garlic. J. Am. Chem. Soc. 67, 1032–1033.

Colangeli, R., Helb, D., Sridharan, S., Sun, J., Varma-Basil, M., Hazbon, M.H., Harbacheuski, R., Megjugorac, N.J., Jacobs Jr., W.R., Holzenburg, A., Sacchettini, J.C., Alland, D., 2005. The *Mycobacterium tuberculosis iniA* gene is essential for activity of an efflux pump that confers drug tolerance to both isoniazid and ethambutol. Mol. Microbiol. 55, 1829–1840.

Gibbons, S., Udo, E.E., 2000. The effect of reserpine, a modulator of multidrug efflux pumps, on the in vitro activity of tetracycline against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) possessing the tet(K) determinant. Phytotherapy Res. 14, 139–140.

Lavaud, C., Massiot, G., Barrera, J.B., Moretti, C., LeMen-Oliver, L., 1994. Triterpene saponins from *Myrsine pellucida*. Phytochemistry 37 (6), 1671–1677.

Mimaki, Y., Kanmoto, T., Sashida, Y., Nishino, A., Satomi, Y., Nishino, H., 1996. Steroidal saponins from the underground parts of *Chlorophytum comosum* and their inhibitory activity on tumour promotor-induced phospholipids metabolism of HeLa cells. Phytochemistry 41, 1405–1410.

Muñoz, O., Piovano, M., Garbaino, J., Hellwing, V., Breitmaier, E., 1996. Tropane alkaloids from *Schizanthus litoralis*. Phytochemistry 43 (3), 709–713.

Sander, P., De Rossi, E., Boddinghaus, B., Cantoni, R., Branzoni, M., Bottger, E.C., Takiff, H., Rodriquez, R., Lopez, G., Riccardi, G., 2000. Contribution of the multidrug efflux pump LfrA to innate mycobacterial drug resistance. FEMS Microbiol. Lett. 193, 19–23.

Scholze, A., Loddenkemper, C., Grünbaum, M., Moosmayer, I., Offermann, G., Tepel, M., 2005. Cutaneous *Mycobacterium abscessus* infection after kidney transplantation. Nephrol. Dialysis Transplant. 20 (8), 1764–1765.

Seeram, N.P., Jacobs, H., McLean, S., Reynolds, W.F., 1998. A prenylated benzopyran derivative from *Peperomia clusifolia*. Phytochemistry 49 (5), 1389–1391.

Sermet-Gaudelus, I., Le Bourgeois, M., Pierre-Audigier, C., Offredo, C., Guillemot, D., Halley, S., Akoua-Koffi, C., Vincent, V., Sivadon-Tardy, V., Ferroni, A., Berche, P., Scheinmann, P., Lenoir, G., Gaillard, J.L., 2003. *Mycobacterium abscessus* and children with cystic fibrosis. Emerg. Infect. Dis. 9, 1587–1591.

Tabuti, J.R.S., Lye, K.A., Dhillion, S.S., 2003. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. J. Ethnopharmacol. 88, 19–44.

Tandon, M., Shukla, Y.N., Thakur, R., 1992. 4-Hydroxyl-8,11-oxidoheneicosanol and other constituents from *Chlorophytum arundinaceum* roots. Phytochemistry 31 (7), 2525–2526.