Phytochemistry, Vol. 28, No. 9, pp. 2439–2441, 1989.
Printed in Great Britain.

0031–9422/89 \$3.00+0.00

MULTIFIDOL AND MULTIFIDOL GLUCOSIDE FROM THE LATEX OF *JATROPHA MULTIFIDA**

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(*Received* 19 *December* 1988)

Key Word Index—*Jatropha multifida*; Euphorbiaceae; latex; acylphloroglucinols; isolation and structural elucidation; multifidol; multifidol glucoside.

Abstract—Two naturally occurring and immunologically active acylphloroglucinols (named multifidol and multifidol glucoside) have been isolated from the latex of *Jatropha multifida* and identified as (2-methylbutyryl)phloroglucinol and 1-[(2-methylbutyryl)phloroglucinyl]-β-D-glucopyranoside.

INTRODUCTION

Jatropha multifida L. (Euphorbiaceae) a shrub of 2 to 3 m in height is endemic in South America, but is widely grown for its ornamental foliage and flowers in southeast Asia [2]. The latex is used in folkloric medicine for the treatment of infected wounds, skin infections, scabies and is commonly known as 'Jodium' in East Java (Surabaya). Its importance as a medicine is reflected by many names: 'jarak gurita' or 'jarak cina' (Indonesia), 'mana' (Philippines), 'manaku' (Malaysia), 'kuthinervala' (Tamil), and 'bhadradanti' (Sanskrit) [3–6].

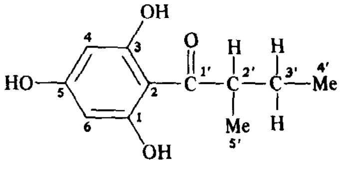
Although the curative properties of the latex have been known for generations, few literature data are available on the chemistry of the latex [7]. To verify the empirical data on its therapeutic effect, an immunomodulatory activity guided isolation [8] of the latex constituents was established. In this paper we report on the isolation and structure of two closely related acylphloroglucinol derivatives.

RESULTS AND DISCUSSION

The immunomodulatory activity-guided isolation of the 20% methanol fraction resulted in two compounds **1** and **2**, both of which show a strong inhibition of the chemiluminescence (assay according to Allen [9]) and the classical pathway complementary activity (microtiter assay described by Klerx *et al.* [10]). A full paper with data on immunological activities of these two components will be published elsewhere. A preliminary report of these activities was made recently [11].

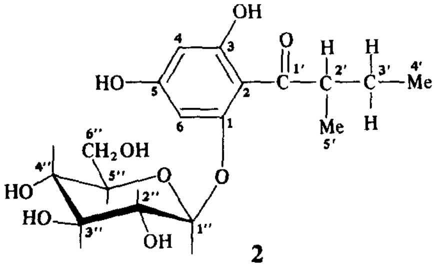
Compound **1** was identified as (2-methylbutyryl)phloroglucinol. Its ^{1}H NMR spectrum in pyridine-d_5 is identical with the synthetic (*S*)-(+)-2-methylbutyryl)phloroglucinol. The racemic mixture of this compound, however, has an identical spectrum [12]. Direct comparison with a synthetic compound prepared by Crombie *et al.* [13] was not possible because of resinification of that material (Crombie, L., personal communication). As the CD spectrum exhibits a positive

Multifidol
(2-methylbutyryl) phloroglucinol



1

Multifidol glucoside 1-[(2-methylbutyryl) phloroglucinyl]-β-D-glucopyranoside



2

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*Part of this work has been presented at the 36th annual meeting of the 'Gesellschaft für Arzneipflanzenforschung', held in September 1988 in Freiburg, F.R.G. [1].

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Cotton effect with only one maximum at 280 nm, most likely the absolute configuration for all or most of the material of compound **1** is the (2*S*)-(+)-configuration.

The glucose moiety of compound **2** is identified as β-D-glucosyl by means of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) [14]. The attachment of the glucosyl moiety at C_1-OH is strongly indicated by its ^{13}C and ^{1}H chemical shifts of C4 and C6 and C_4H and C_6H respectively. In compound **1** these chemical shifts appear as one singlet (^{13}C: δ94.7; ^{1}H: δ5.81) due to the symmetry of its aromatic moiety. In compound **2**, however, these carbons and protons are not identical any more; (^{13}C: δ95.0 and 97.2; ^{1}H: δ5.85 and 6.07 respectively).

The attachment of the glucosyl moiety at C_1-OH is proved by ^{1}H NOESY experiments. There is a correlated cross-relaxation between the anomeric $C_1''H$ and the aromatic C_6H and not with C_4H.

β-Glucosidase, an enzyme which splits off specifically β-D-glucose from mono-β-glucosides [15], was not able to hydrolyse compound **2**, probably because of steric hindrance of the aglucone-moiety.

The ^{13}C NMR spectrum show two pairs of chemical shifts for most of the aglucone carbons, the intensities of one pair being about 1/5th of the other. The CD-spectrum exhibits a positive Cotton effect at 280 nm (the same as compound **1**) and a negative Cotton effect at 310 nm, indicating that the aglucone moiety for most of compound **2** has the same absolute configuration (2*S*) as compound **1**. To our knowledge compound **2** has not been described in the literature, whereas compound **1** has not been described as a natural compound.

EXPERIMENTAL

Plant material. Latex was collected from the leaf-stalks of two-year-old plants of *J. multifida*, cultivated in the garden of the first author in Surabaya (Indonesia). To that end young stalks were cut at two weeks intervals at 5 a.m. from December to March 1987. The harvested crude latex was kept at −20°. Plants were also cultivated in the green house of Utrecht University, Netherlands and in the botanical garden of Santo Tomas University, The Philippines, from seeds of the Surabaya population.

Isolation. Crude latex was mixed with an equal amount of H_2O, centrifuged and the supernatant was freeze-dried yielding a solid which was *ca* 10% of the latex. 500 mg dried H_2O soluble material was chromatographed on a polyamide column (4.5 × 25 cm, MN-Polyamide-CC 6, grain size 0.16 μm) successively with 500 ml of the following mixtures MeOH–H_2O, (0:100), (1:4), (2:3), (3:2) and (100:0) as eluting solvents, yielding 161, 30, 93, 42 and 16 mg respectively. Prep. TLC of the concd MeOH–H_2O (1:4) fraction (30 mg) on two silica gel 60 TLC plates, F-254 (1 mm) using $CHCl_3$–MeOH–H_2O (13:10:2) as solvent, yielded two compounds (visualized under UV 254 nm): **1** (R_f 7.1) and **2** (R_f 5.4), of which 7 and 13 mg was recovered with MeOH respectively.

Compound **1** $C_{10}H_{15}O_4$ FAB-MS: $(M+H)^+$ (*m/z* calcd 211.0977, detd 211.0970), mp 116–118°, 7 mg. UV λ_{max}^{MeOH} nm (log ε): 229 (3.66), 287 (3.81); UV $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 219 (3.71), 312 (3.92). CD (MeOH; *c* 4.76.10^{-4}) $[\theta]^{20}$(nm): +311 974 (280). IR ν_{max}^{KBr} cm^{-1}: 3302 (40.3) (OH), 1605 (14.0) (C=O). ^{1}H NMR (DMSO-d_6): see Table 1; ^{13}C NMR (DMSO-d_6): see Table 2.

Compound **2**. $C_{17}H_{25}O_9$ FABMS $(M+H)^+$ (*m/z* calcd 373.1475 detd 373.1498), mp 139–140°, 13 mg. UV λ_{max}^{MeOH} nm (log ε): 225 (3.97), 287 (4.20); UV $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 219 (4.15), 312 (4.33). CD (MeOH; *c* 2.69.10^{-4}) $[\theta]^{20}$ (nm): +429 368 (280), −208 550 (310). IR ν_{max}^{KBr} cm^{-1}: 3389 (22.6) (OH), 1602 (13.2) (C=O). ^{1}H NMR (DMSO): see Table 1; ^{13}C NMR (DMSO and CD_3OD) see Table 2. Hydrolysis of compound **2** with 2% trifluoroacetic acid afforded compound **1** and glucose, which were identified on TLC using $CHCl_3$–MeOH–H_2O (13:10:2) as solvent on silica gel. After incubation at 35° of compound **2** on a silica gel TLC plate with β-glucosidase (0.25 mg/ml) in Pi buffer pH 4.5 for 15 min no hydrolysis took place; this is in contrast with the reference compound emodin-β-monoglucoside.

Table 1. ^{1}H NMR assignment compounds for **1** and **2** (300 MHz)

H	**1** (DMSO) δ	Coupled to (COSY)	**2** (DMSO) δ	Coupled to H (COSY)
4	5.81 2H, *s*		5.85, 1H, *br s*	6*
6	5.81		6.07, 1H, *d* (J = 1.9)	4*
2′	3.75, 1H, *m*	5′,3′A,3′B	3.77, 1H, *m*	5′,3′A,3′B
3′A	1.71, 1H, *m*	2′,3′B,4′	1.70, 1H, *m*	2′,3′B,4′
3′B	1.28, 1H, *m*	2′,3′A,4′	1.31, 1H, *m*	2′,3′A,4′
4′	0.81, 3H, *t*	3′A,3′B	0.83, 3H, *t*	3′A,3′B
5′	1.03, 3H, *d* (J = 6.7)		1.04, 3H, *d* (J = 6.7)	
1″			4.89, 1H, *d* (J = 6.8)	2″
2″			*ca.* 3.28 1H, *m*	
3″ + 4″			*ca.* 3.24, 2H, *m*	
5″			*ca.* 3.27, 1H, *m*	
6″A			3.70, 1H, *br d* (J = 11.4)	
6″B			3.51, 1H, *dd* (J = 11.9; 4.9)	

*Long range coupling.

Table 2. ^{13}C NMR assignment for compounds **1** and **2** (100.6 MHz)

	^{13}C assignments		^{13}C assignments from 2D spectra		
	1	**2**		Coupled to H 2D-^{13}C–^{1}H NMR	
	DMSO	DMSO	CD_3OD		
C	δ	δ	δ	Short range	Long range
1	164.7	160.2 160.1†	161.7		6 + 1''
2	104.5	104.3 104.6†	106.5		4 + 6
3	164.7	165.4 165.3†	166.6		n.d.
4	94.7	95.0	95.7	4	4 + 6
5	165.0	166.7	167.4		4 + 6
6	94.7	97.2	98.6	6	4 + 6
1'	208.5	208.6 208.7†	211.7		5'
2'	44.5	44.8 44.9†	46.8	2'	4' + 5'
3'	26.5	26.6 26.1†	28.2	3'A + 3'B	4'
4'	11.9	11.5	12.0	4'	—
5'	16.7	16.8 17.4†	16.8	5'	—
1''		100.3	101.5	1''	1'' + 2''
2''		73.3	74.7	2''	2'' + 3''?
3''		77.1*	78.6	3''	2''? + 3'' + 4''
4''		69.5	71.1	4''	3''? + 4'' + 5'' + 6''B
5''		77.0*	78.2	5''	4'' + 5'' + 6''B
6''		60.5	62.4	6''A + 6''B	6''B + 5''

*Assignments with the same exponent may be interchanged.
†Chemical shifts of minor intensity (*ca* 20%).
n.d.: not detected.

Acknowledgements—This study was financed by BIOHORMA, Elburg, The Netherlands. The authors acknowledge this support gratefully. The authors would like to thank Mr B. Lutz (Analytisch Chemisch Laboratorium, Utrecht), Mr C. Versluis (Analytisch Chemisch Laboratorium, Utrecht), and Mr C. Erkelens (Gorlaeus Laboratoria, Leiden) for the recording and discussion of IR, mass and NMR spectra respectively.

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