Pergamon 0031-9422(1995)00300-2 Phytochemistry, Vol. 40, No. 2, pp. 597–598, 1995

0031 9422/95 $9.50 + 0.00

MULTIFIDIN—A CYANOGLUCOSIDE IN THE LATEX OF *JATROPHA MULTIFIDA*

ALBERT J. J. VAN DEN BERG,* STEPHAN F. A. J. HORSTEN, J. JANTINA KETTENES-VAN DEN BOSCH,† BURT H. KROES and RUDI P. LABADIE

Department of Pharmacognosy, Faculty of Pharmacy, Universiteit Utrecht, P.O. Box 80082, 3508 TB Utrecht, The Netherlands; †Department of Pharmaceutical Analysis, Faculty of Pharmacy, Universiteit Utrecht, The Netherlands

(*Received* 30 *January* 1995)

Key Word Index — *Jatropha multifida*; Euphorbiaceae; cyanoglucoside; multifidin; 1-cyano-3-β-D-glucopyranosyloxy-(*Z*)-1-methyl-1-propene.

Abstract — A novel non-cyanogenic cyanoglucoside, 1-cyano-3-β-D-glucopyranosyloxy-(*Z*)-1-methyl-1-propene, was isolated from the latex of *Jatropha multifida*. The compound was named multifidin.

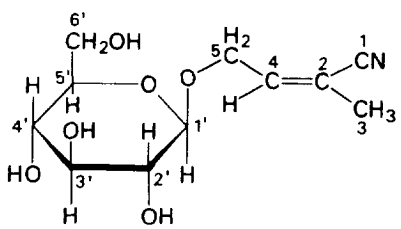
INTRODUCTION

Cyanogenesis is a well-known phenomenon in many Euphorbiaceous plants. Within the Euphorbiaceae, several cyanogenic glucosides have been found: in the subfamily Phyllanthoideae, triglochinin, dhurrin, and taxiphyllin occur, all three being derived from tyrosine. The cyanogenic glycoside acalyphin, a nicotinic acid derivative, is found within the Acalyphoideae, whereas linamarin and lotaustralin which arise from valine and isoleucine, respectively, have been identified in Crotonoideae [1, 2]. Cyanogenic properties have been reported for *Jatropha angustidens*, *J. capensis*, *J. hieronymi* and *J. macrocarpa* (Crotonoideae), but their constituents leading to cyanogenesis are not known [1, 3]

Previously, we reported the isolation of two cyclic peptides, as well as a phloroglucinol derivative (i.e. multifidol) and its glucoside, from the latex of *Jatropha multifida* L. [4, 5]. The present communication deals with the structure elucidation of a novel non-cyanogenic nitrile-containing glucoside, which was also isolated from the latex mentioned above.

RESULTS AND DISCUSSION

Compound **1** was isolated from the latex of *J. multifida*. Its FAB mass spectrum showed an $[M + H]^+$ at *m/z* 260 indicating a molecular mass of 259, and thus the presence of one nitrogen. The ^1H and ^{13}C NMR spectra (including an APT and two-dimensional ^1H COSY and HETCOR spectra) in DMSO-d_6 showed the presence of a glucose moiety; the coupling constant of the anomeric proton (H-1′, δ 4.18, *J* = 7.8 Hz) indicated a β-glucosidic linkage. The proton-decoupled ^{13}C NMR spectrum of **1** showed 11 signals, six of which were assigned to a β-D-glucopyranoside unit [6]. TLC analysis of the acid hydrolysate of **1** also indicated the presence of glucose.



1

In the ^1H NMR spectrum four resonances were observed in addition to those assigned to glucose, i.e. a multiplet at δ 6.45 [1H, H-4, *J*(4-5A) = *J*(4-5B) = 6.8 Hz, *J*(4-3) ≈ 1.5 Hz], two multiplets at δ 4.40 and 4.27 [2H, H-$5_{A,B}$, *J*(5A-5B) = 13.5 Hz, *J*($5_{A,B}$-3) ≈ 1 Hz] and a multiplet at δ 1.96 (3H, methyl group, H-3). Of the five remaining signals in the ^{13}C NMR spectrum, the APT and HETCOR spectra showed one signal to belong to a methyl group (δ19.4, C-3; ^1H at δ1.96), one to a methine carbon (δ144.3, C-4; ^1H at δ6.45), one to a methylene carbon (δ66.5, C-5; ^1H at 4.40 and 4.27), and two to quaternary carbons (δ117.3, C-1 and 110.4, C-2). Combination of the data mentioned above resulted in the molecular formula $C_{11}H_{17}NO_6$.

The IR spectrum showed a sharp absorption band at 2225 cm^{-1}, characteristic of a nitrile group (^{13}C signal at δ117.3). The fact that the nitrile absorption is observed in the IR spectrum, indicates the absence of oxygen at the nitrile-bearing carbon; in cyanogenic α-hydroxynitrile (cyanohydrin) glycosides the nitrile band is quenched [7].

*Author to whom correspondence should be addressed.

In keeping with this compound **1** does not show cyanogenic properties. Thus incubation of **1** with β-glucosidase (ex. almonds) in phosphate buffer pH 7 at 37° for 24 hr did not result in any formation of HCN (Guignard detection with sodium picrate paper). The IR spectrum showed also a band at 1640 cm^{-1} which can be assigned to a double bond. The corresponding ^{13}C signals are found at δ 144.3 (CH) and 110.4 (quaternary C).

Based on these spectral data, we assign structure **1** to the glycoside. The stereochemistry of the olefinic bond was determined as (*Z*) in a NOE difference experiment. Upon irradiation of the methyl resonance and after subtraction of the non-methyl irradiated spectrum, all resonances were cancelled with the exception of the signal due to the vinylic proton at C-4. This indicates proximity of the vinylic proton and methyl protons. Therefore, we concluded that compound **1** is 1-cyano-3-β-D-glucopyranosyloxy-(*Z*)-1-methyl-1-propene, and propose the name multifidin. Multifidin is an isomer of 1-cyano-3-β-D-glucopyranosyloxy-2-methyl-1-propene which was recently isolated from the epidermis of *Hordeum vulgare* [8]. Regarding its structure, multifidin is likely to be derived from isoleucine. This would correspond with the biosynthesis of cyanogenic glycosides in Crotonoideae (including the genus *Jatropha*), which most probably proceeds through the valine/isoleucine pathway [1].

EXPERIMENTAL

Plant material. Jatropha multifida L. Plants grown in Jepara (near Kudus, Java, Indonesia) were identified by comparison with authentic plants in Bogor Botanical Gardens. Crude latex was obtained by cutting off the leaf stalks and adding a few drops of EtOH to suppress excessive foaming. The latex was stored at −20° until use.

Isolation procedure. Crude latex (50 ml) was mixed with 200 ml demineralized H_2O and 2 ml EtOH, and subsequently extracted with 3 × 500 ml EtOAc. EtOAc was removed from the extract under reduced pressure. The residue was dissolved in 1 ml MeOH and fractionated on Sephadex LH-20 (Pharmacia; column dimensions: 1.4 cm i.d. × 40 cm) with MeOH as eluting solvent at a flow rate of 0.35 ml/min; fractions of 8 ml were collected. Fractions containing **1** were identified by TLC on silica gel using $CHCl_3$–MeOH–H_2O, (5:4:1); compound **1** was detected with I_2. Frs containing **1** were combined, and further fractionated by prep. CC on silica gel H (Merck, no. 7736) using a Miniprep LC apparatus (Jobin Yvon, France; column dimensions: 1.4 cm i.d. × 40 cm; column pressure 8 bar) with cyclohexane–*iso*-PrOH–MeOH (5:5:2) as eluting solvent (solvent pressure 2 bar); fractions of 15 ml were collected. Fractions containing only **1** were collected to yield 42 mg after evaporation of the solvent under N_2.

Compound **1**. $C_{11}H_{17}NO_6$. ^1H and ^{13}C NMR spectra (including ^1H COSY, APT and HETCOR spectra) were recorded at 298 K with a Bruker MSL-400 instrument; the chemical shifts are reported in ppm relative to TMS. A NOE difference spectrum in DMSO-d_6 was recorded with a Gemini-300 MHz instrument (Varian) at 298 K.

^1H NMR (400 MHz, DMSO-d_6 with 5% D_2O): δ6.45 [*m*, H-4, *J*(4-5A) = 6.8 Hz, *J*(4-5B) = 6.8 Hz, *J*(4-3) ≈ 1.5 Hz], 4.40 [*m*, H-5A, *J*(5A-5B) = 13.5 Hz, *J*(5A-4) = 6.8 Hz, *J*(5A-3) ≈ 1 Hz], 4.27 [*m*, H-5B, *J*(5B-5A) = 13.5 Hz, *J*(5B-4) = 6.8 Hz, *J*(5B-3) ≈ 1 Hz], 4.18 [*d*, H-1′, *J*(1′ − 2′) = 7.8 Hz], 3.65 [*dd*, H-6′A, *J*(6′A-6′B) = 11.8 Hz, *J*(6′A-5′) = 2.0 Hz], 3.44 [*dd*, H-6′B, *J*(6′B-6′A) = 11.8 Hz, *J*(6′B-5′) = 5.7 Hz], 3.15 [*t*, H-3′, *J*(3′ − 2′) ≈ 9 Hz, *J*(3′ − 4′) ≈ 9 Hz], 3.10 [*m*, H-5′], 3.05 [*t*, H-4′, *J*(4′ − 3′) ≈ *J*(4′ − 5′) ≈ 9 Hz], 2.96 [*t*, H-2′, *J*(2′ − 1′) = 7.8 Hz, *J*(2′ − 3′) ≈ 9 Hz], 1.96 [H-3, *m*, *J*(3-5A) ≈ 1 Hz, *J*(3-5B) ≈ 1 Hz, *J*(3-4) ≈ 1.5 Hz]; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 144.3 (C-4), 117.3 (C-1), 110.4 (C-2), 102.6 (C-1′), 76.9 (C-5′), 76.6 (C-3′), 73.3 (C-2′), 69.9 (C-4′), 66.5 (C-5), 60.9 (C-6′), 19.4 (C-3); FAB-MS (matrix *m*-nitrobenzyl alcohol) *m/z*: 260 $[M + H]^+$; IR ν_{max} (KBr) cm^{-1}: 3410 (–OH), 2225 (–C≡N), 1640 (>C = C<), and 1110–1020 (C–O). Hydrolysis of compound **1** in a sealed vial in 2 M TFA at 110° for 4 hr yielded glucose which was identified by TLC on silica gel using *n*-BuOH–Me_2CO–H_2O (4:5:1; saturated chamber) as solvent system and naphthoresorcinol–H_2SO_4 (105° for 10 min) for detection.

Acknowledgements—The authors are grateful to Mrs A. C. H. T. M. van der Kerk-van Hoof (Analytical Molecular Spectrometry, Universiteit Utrecht) for recording the FAB mass spectrum, and Dr C. Erkelens (NMR Department, Gorlaeus Laboratories, State University of Leiden) for recording the Bruker NMR spectra.

REFERENCES

1. Hegnauer, R. (1989) *Chemotaxonomie der Pflanzen — Band* 8, pp. 446–447. Birkhäuser, Basel.
2. Hegnauer, R. (1986) *Chemotaxonomie der Pflanzen — Band* 7, p. 356. Birkhäuser, Basel.
3. Hegenauer, R. (1966) *Chemotaxonomie der Pflanzen — Band* 4, p. 125. Birkhäuser, Basel.
4. Kosasi, S., Van der Sluis, W. G., Boelens, R., 't Hart, L. A. and Labadie, R. P. (1989) *FEBS Letters* **256**, 91.
5. Kosasi, S., Van der Sluis, W. G. and Labadie, R. P. (1989) *Phytochemistry* **28**, 2439.
6. Bock, K. and Pedersen, C. (1983) *Adv. Carbohydr. Chem. Biochem.* **41**, 27.
7. Seigler, D. S. (1977) *Prog. Phytochem.* **4**, 83.
8. Pourmohseni, H., Ibenthal, W.-D., Machinek, R., Remberg, G. and Wray, V. (1993) *Phytochemistry* **33**, 295.