Pergamon S0031-9422(96)00018-0 Phytochemistry, Vol. 42, No. 3, pp. 633–636, 1996

0031-9422/96 $15.00 + 0.00

A CYANOGENIC GLYCOSIDE FROM *CANTHIUM SCHIMPERIANUM*

BRUNHILDE SCHWARZ, VICTOR WRAY* and PETER PROKSCH†

Lehrstuhl für Pharmazeutische Biologie, Julius-von-Sachs-Institut für Biowissenschaften, Universität Würzburg, Mittlerer Dallenbergweg 64, D-97082 Würzburg, Germany; *Gesellschaft für Biotechnologische Forschung mbH, Marscheroder Weg 1, D-38124 Braunschweig, Germany

(*Received in revised form* 14 *November* 1995)

Key Word Index—*Canthium schimperianum*; Rubiaceae; cyanogenic glycoside; structural elucidation; 3,4-dicaffeoylquinic acid; 3,5-dicaffeoylquinic acid; *Spodoptera littoralis*.

Abstract—A new cyanogenic glycoside esterified with an iridoid glycoside, 2*R*-[(2-methoxybenzoylgenoposidyl)-5-*O*-*β*-D-apiofuranosyl-(1 → 6)-*β*-glucopyranosyloxy]-2-phenyl acetonitrile, was isolated from the seeds of *Canthium schimperianum* and identified from its spectroscopic data. The new compound showed weak growth retarding activity towards neonate larvae of *Spodoptera littoralis* (ED_{50} 8580 ppm) compared to the co-occurring hydroxycinnamic acids, 3,4-dicaffeoylquinic acid (ED_{50} 550 ppm) and 3,5-dicaffeoylquinic acid (ED_{50} 520 ppm).

INTRODUCTION

As part of a broad survey of secondary plant compounds that influence seed dispersal by birds, as well as seed predation by herbivorous insects, we became interested in the fruits of *Canthium schimperianum Lam.* because they show growth retarding activity against larvae of the polyphagous herbivorous insect *Spodoptera littoralis* (Noctuidae). Bioassay guided fractionation of an extract obtained from air dried fruits of *C. schimperianum* yielded a new cyanogenic glycoside (**1**) which was identified by spectroscopic means. Further secondary compounds with growth retarding activity towards neonate larvae of *S. littoralis* included 3,4- and 3,5-dicaffeoylquinic acid.

RESULTS AND DISCUSSION

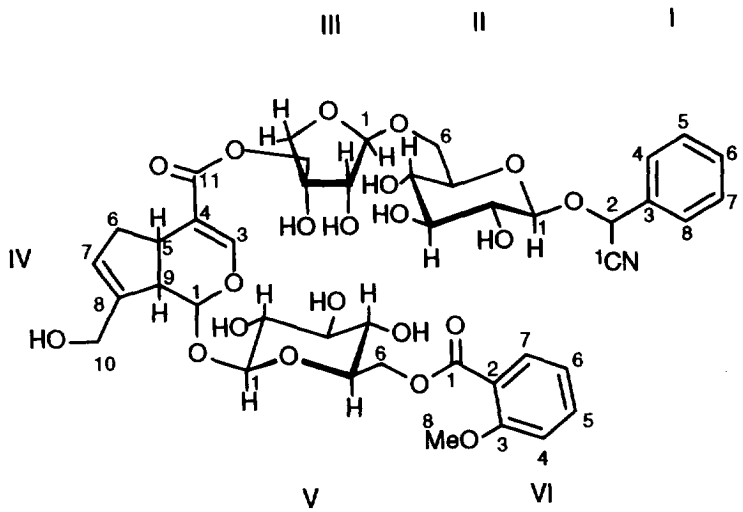
The new cyanogenic glycoside **1** was isolated from the methanol extract of air dried fruits of *C. schimperianum* by column chromatography on Sephadex LH20 (100% methanol) and RP-18 (30% methanol).

The structure of **1** was deduced from its NMR and mass spectral data. Its positive ion FAB mass spectrum contained a molecular ion peak at *m/z* 918 $[M + H]^+$ that is compatible with the molecular formula $C_{43}H_{51}NO_{21}$. The ^1H 1D and 2D COSY spectra allowed the nature of the various fragments in the molecule to be characterized. The cross peaks in the COSY spectra for eight aromatic protons arose from mono- and di-substituted benzenes. Anomeric signals of three sugar moieties were identified in the region *δ* 4.7 to 5.1. Two of these were from *β*-glucopyranose systems (**1**, fragments II and V). The third was a little unusual and arose from a pentose system (fragment III). This was established to be an apiose system and has been found by us in other studies [1, 2]. It was recognized from its characteristic shifts and the presence of spin systems corresponding to isolated methylenoxy groups (also see below). Finally, the fragment $OCH(O)CHCHCH_2CH{=}C(CH_2O)$ or $OCH(O)CHCHCH_2C(CH_2O){=}CH$ belonging to the iridoid fragment in the final structure (fragment IV) was evident in the COSY spectrum.

Useful additional structural information is usually provided by careful inspection of the weaker cross peaks in the COSY spectrum that arise from long-range ^1H–^1H coupling. Thus, allylic and homoallylic couplings in the iridoid fragment were observed for the two methylene groups, although at this stage the oxygen-carrying group could not be unambiguously positioned. Further couplings from several protons in this fragment were found to an additional singlet low-field olefinic proton that overlapped with the aromatic protons. These correspond to couplings from H-5 and H-1 to H-3 (**1**). In addition, the aromatic protons showed several diagnostic cross peaks. Couplings were observed between the methoxyl group and an *ortho* proton of the disubstituted benzene, and between the low-field singlet at *δ* 5.84 and the *ortho* protons of the phenyl system (corresponding to H-2^I and H-$4^I/8^I$, **1** and Table 1).

In the next step, all protonated carbons were assigned from inspection of the cross peaks in the HMQC spectrum, arising from one-bond correlations between ^1H and ^{13}C. A 2D ^1H-detected multiple-bond ^{13}C–^1H correlation that showed cross peaks for through-bond correlations over two and three bonds afforded suffi-

†Author to whom correspondence should be addressed.



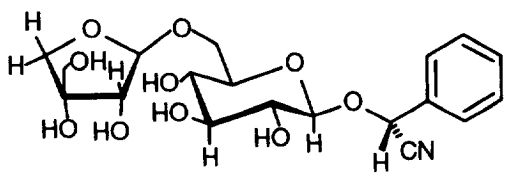
1

cient information to confirm the nature of each of the above fragments and, more importantly, provided the necessary correlations for delineating their sequence. Only selected intra-fragment correlations are reported in Table 1, others were observed that confirmed the unambiguous fragments above and hence are not documented. The position of the $HOCH_2$ group in the iridoid fragment follows directly from the three-bond correlations of H-1, H-9 and H-10A/B. Similarly, the correlations of H-1 and H-3 established the substitution pattern of the heterocyclic ring with the olefinic proton adjacent to the ring oxygen. This is rather nicely confirmed by the large characteristic ^1J(CH) of 192 Hz, which is very similar to the equivalent couplings in dihydro-1,4-dioxin and 1,4-benzodioin [3].

The long-range $^{13}C-^1H$ correlations for the pentose fragment confirmed the furanose form of the ring (from correlations of H-1^{III} and H-$4B^{III}$) and the position of the external CH_2O group at C-3^{III} (from correlations of H-$5A^{III}$/$5B^{III}$). Of course, these do not establish the stereochemistry of the ring substitutents. The close correspondence of the ^{13}C shifts (C-1 to C-5: δ 110.6, 78.5, 78.9, 75.0 and 66.6) with those reported previously [2] (C-1 to C-5: δ 108.9, 78.5, 78.8, 75.1 and 67.4 ppm) was strong evidence that we were dealing with a β-apiofuranosyl system. The observation of only one correlation to the quaternary nitrile carbon of the cyanogenic glycoside moiety is expected. Unfortunately, it is not possible to determine unambiguously the absolute stereochemistry at C-2^I as only small differences were observed in the ^{13}C chemical shifts for this carbon and C-1^I and C-1^{II} of the adjacent glucose moiety between the *R* and *S* isomers [4]. However, the subtle differences between the shifts reported here and

Table 1. I–VI corresponds to fragments I–VI in formula **1**

Intra-fragment correlations			
Apiose fragment (III)		Iridoid fragment (IV)	
Proton coupled carbons		Proton coupled carbons	
H-1^{III}	C-3^{III}, C-4^{III}	H-1^{IV}	C-3^{IV}, C-5^{IV}, C-8^{IV}, C-9^{IV}
H-$4B^{III}$	C-1^{III}, C-3^{III}	H-9^{IV}	C-1^{IV}, C-5^{IV}, C-6^{IV}, C-8^{IV}, C-10^{IV}
H-$5A^{III}$	C-2^{III}, C-3^{III}, C-4^{III}	H-10A/B^{IV}	C-7^{IV}, C-8^{IV}, C-9^{IV}
H-$5B^{III}$	C-2^{III}, C-3^{III}, C-4^{III}	H-3^{IV}	C-1^{IV}, C-4^{IV}, C-5^{IV}, C-11^{IV}
Phenyl fragment (I)			
Proton coupled carbons			
H-2^I	C-1^I, C-3^I, C-4^I/8^I		
Inter-fragment correlations			
Cyanogenic glucoside moiety		Iridoid glucoside moiety	
Proton coupled carbon		Proton coupled carbon	
H-2^I	C-1^{II}	H-$6A^V$	C-1^{VI}
H-1^{II}	C-2^I	H-$6B^V$	C-1^{VI}
H-6^{II}	C-1^{III}	H-1^V	C-1^{IV}
H-1^{III}	C-6^{II}	H-1^{IV}	C-1^V
H-$5A^{III}$	C-11^{IV}		
H-$5B^{III}$	C-11^{IV}		



2

those found by Schwind *et al.* [2] in methanol for xeranthin, which contains a 2*S*-β-D-glucopyranosyl-2-(3-hydroxy)phenyl acetonitrile moiety, tend to favour the *R*-configuration at C-2[1] in the present compound. This configuration has also been found for oxyanthin (**2**), which has been identified in the Rubiaceae as the first cyanogenic glucoside documented in the order Gentianales [1]. Compound **2** is the second naturally occurring cyanogenic glycoside bearing an apiose moiety besides xeranthin [1]. The new cyanogenic glycoside was only found in fruits (3.5% dry wt) of *C. schimperianum*. Within the fruits, the cyanogenic glycoside is exclusively confined to the seeds. The extract of fruits of *C. schimperianum* was incorporated into an artificial diet and tested for insecticial activity towards neonate larvae of *S. littoralis*. After six days of exposure, larval survival and larval weight were monitored and compared to controls. The extract of *C. schimperianum* caused significant reduction of the larval weight (Table 2). None of the neonate larvae, however, died during the experiment. Bioassay guided isolation afforded 3,5- and 3,4- dicaffeoylquinic acid (identified by NMR and mass spectra [5] and the new cyanogenic glycoside **1**. When incorporated into artificial diet and offered to neonate larvae of *S. littoralis* in a chronic feeding experiment over a period of six days, the two dicaffeoylquinic acids were similar with regard to growth retarding activity. The ED_{50} of 3,4-dicaffeoylquinic acid was 550 ppm (0.97 $\mu g\, g^{-1}$ fr. wt) and the ED_{50} of 3,5-dicaffeoylquinic acid was 420 ppm (0.81 $\mu g\, g^{-1}$ fr. wt). The new cyanogenic glycoside (**1**) showed only weak growth retarding activity against the herbivore *S. littoralis*; the ED_{50} of the latter compound was 8580 ppm (9.35 $\mu g\, g^{-1}$ fr. wt).

EXPERIMENTAL

General. NMR: 300 K on a Bruker DMX 600 (1D: ^1H and ^{13}C spectra, 2D: COSY, ^1H-detected one-bond [6] and multiple-bond ^{13}C multiple-quantum coherence spectra [7], HMQC and HMBC), spectrometer locked to the major deuterium resonance of the solvent, CD_3OD. IR: KBr pellets; FAB-MS: glycerol as matrix. Frs were monitored by TLC (UV 254 nm), solvent: EtOAc–HCO_2H–HOAc–H_2O (100:11:11:27).

Isolation. Ripe fruits of *C. schimperianum* were collected in the Comoe National Park (Ivory Coast) in November 1992 and identified by Dr Piere Poilecot. Air dried fruits were extracted with MeOH. After evapn of the solvent, the residue was suspended in H_2O and extracted with EtOAc. The H_2O extract was dried and subjected to Sephadex LH20 CC (100% MeOH) and RP-18 CC (30% MeOH) to give 1.09 g (3.5%) of **1**. The EtoAc extract was subjected to RP-18 CC (30% MeOH) to give 22.7 mg (0.07%) 3,5-dicaffeoylquinic acid and 40.5 mg (0.13%) 3,4-dicaffeoylquinic acid, which were identified by ^1H and ^{13}C NMR and mass spectroscopy [5].

Compound. Amorphous pale-yellow powder, $[\alpha]_D^{20}$ −39.2 (MeOH, *c* 1.0). UV λ_{max} 238 (0.9), 298 (0.2); FAB-MS *m/z*: 918, 792, 623, 153; IR ν_{max}^{KBr} cm^{-1}: 3424, 2916, 1708, 1630, 1601, 1492, 1458, 1438, 1252; ^1H and ^{13}C NMR: Table 3.

Experiments with insects. Larvae of *S. littoralis* were from a laboratory colony reared on an artificial diet under controlled conditions as described previously [8]. The chronic feeding bioassays were conducted with neonate larvae that were kept on diet spiked with extracts, frs or different concs of the compounds studied. After 6 days, survival and larval wt were monitored and compared to controls.

Acknowledgements—We are indebted to T. Hovestadt (University Würzburg) for providing the plant material,

Table 2. Neonate larvae of *Spodoptera littoralis* (n = 20) were released on the treated diet. After 6 days, survival and weight were measured and compared to the control

Species of compound	Dose (ppm)	Survival (% rel. to controls)	Weight (% rel. to controls)
C. schimperianum, fruits	84600	100	4.5
C. schimperianum, leaves	49142	100	53.7
3,4-Dicaffeoylquinic acid	285	100	89.1
3,4-Dicaffeoylquinic acid	571	100	48.3
3,4-Dicaffeoylquinic acid	857	100	23.8
3,5-Dicaffeoylquinic acid	285	100	71.8
3,5-Dicaffeoylquinic acid	571	90	26.3
3,5-Dicaffeoylquinic acid	857	90	24.2
Cyanogenic glycoside **1**	14285	100	37.4
Cyanogenic glycoside **1**	28571	90	20.3
Cyanogenic glycoside **1**	40857	90	11.9

Table 3. ^{1}H and ^{13}C NMR data for compound **1**

C or H	^{13}C	^{1}H	*J* (in Hz)
I			
1	119.3		
2	68.9	5.84 *s*	
3	134.9		
4	128.9	7.63 *m*	
5	130.1	7.50–7.44 *m*	
6	130.9	7.50–7.44 *m*	
7	130.1	7.50–7.44 *m*	
8	128.9	7.63 *m*	
II			
1	102.6	4.39 *d*	(1–2) 7.4
2	74.7	3.32–3.40 *m*	
3	77.8	3.32–3.40 *m*	
4	71.4	3.32–3.40 *m*	
5	77.0	3.32–3.40 *m*	(5–6B) 5.9
6	68.1	6A: 4.11 *dd*	(6A–6B) 10.9
		6B: 3.70 *dd*	
III			
1	110.6	5.12 *d*	(1–2) 1.7
2	78.5	4.07 *d*	
3	78.9		
4	75.0	4A: 4.11 *d*	(4A–4B) 9.9
		4B: 3.89 *d*	
5	66.6	5A: 4.29 *d*	(5A–5B) 11.4
		5B: 4.24	
IV			
1	99.2	4.95 *d*	(1–9) 8.35
3	153.9	7.46 *s*	
4	112.2		
5	36.9	3.16 *ddd*	(5–6A) 8.12
			(5–6B) 8.12
			(5–9) 7.8
6	39.7	6A: 2.76 *dd*	(6A–6B) 15.8
		6B: 1.79 *dd*	
7	128.8	5.66 *dd*	
8	144.8		
9	46.3	2.69 dd	(9–5) 7.8
10	61.5	4.20 2*d*	(10A–10B) 12.8
11	168.6		
V			
1	100.6	4.78 *d*	(1–2) 7.8
2	74.8	3.31 *dd*	(2–3) 9.1
3	77.6	3.50 *dd*	(3–4) 9.1
4	71.9	3.45 *dd*	(4–5) 9.2
5	75.7	3.64 *ddd*	(5–6A) 6.3
6	64.7	6A: 4.57 *dd*	(5–6B) 2.9
		6B: 4.54 *dd*	(6A–6B) 11.8
VI			
1	167.7		
2	120.7		
3	160.7		
4	113.6	7.15 *d*	(5–6) 8.4
5	135.3	7.58 *ddd*	(4–5) 7.4
6	121.3	7.02 *dd*	
7	133.7	7.79 *dd*	(3–4) 7.7
			(3–5) 1.8
8	56.5	3.91 *s*	

S. Harmsen for IR spectra, Dr L. Witte (TU Braunschweig, F.R.G.) for mass spectra, T. Ortman for optical rotation data, and Dr T. Domke (GBF Braunschweig) for his NMR expertise. Financial support by the DFG ('Schwerpunkt: Chemische Ökologie') is gratefully acknowledged.

REFERENCES

1. Rockenbach, J. Nahrstedt, A. and Wray, V. (1992) *Phytochemistry* **31**, 567.
2. Schwind, P., Wray, V. and Nahrstedt, A. (1990) *Phytochemistry* **29**, 1903.
3. Katritzky, A. R., Kingsland, M., Rudd, M. N., Sewell, M. J. and Topsom, R. D. (1967) *Austr. J. Chem.* **20**, 1773.
4. Hübel, W., Nahrstedt, A. and Wray, V. (1981) *Arch. Pharm.* **314**, 609.
5. Kahn, I. A., Tali, T. and Sticher, O. (1993) *Planta Med.* **59**, 288.
6. Bax, A. and Subramanian, S. (1986) *J. Mag. Reson.* **67**, 565.
7. Summers, M. F., Marzilli, L. G. and Bax, A. (1986) *J. Am. Chem. Soc.* **108**, 4285.
8. Srivastava, R. P. and Proksch, P. (1991) *Entomol. Gen.* **15**, 265.