Phytochemistry, Vol. 21, No. 1, pp. 107–110, 1982.
Printed in Great Britain.

0031-9422/82/010107-04 $03.00/0

SARMENTOSIN EPOXIDE, A NEW CYANOGENIC COMPOUND FROM *SEDUM CEPAEA**

ADOLF NAHRSTEDT, AXEL WALTHER and VICTOR WRAY†

Institut für Pharmazeutische Biologie der Technischen Universität, D-3300 Braunschweig, West Germany; †Gesellschaft für Biotechnologische Forschung, D-3300 Braunschweig-Stöckheim, West Germany

(*Received* 31 *March* 1981)

Key Word Index—*Sedum cepaea*; Crassulaceae; cyanogenic compound; sarmentosin epoxide; 4-β-D-glucopyranosyloxy-2,3-epoxy-2-hydroxymethylbutyronitrile.

Abstract—A new cyanogenic compound, sarmentosin epoxide, was isolated from the aerial parts of *Sedum cepaea*. Its structure was established mainly by ^{1}H NMR and ^{13}C NMR methods as 4-β-D-glucopyranosyloxy-2,3-epoxy-2-hydroxymethylbutyronitrile. The new compound is not a typical cyanogenic glucoside but spontaneously releases HCN after hydrolysis of the oxiran group to a cyanohydrin in aqueous solution.

INTRODUCTION

Cyanogenesis in the Crassulaceae has been known for many years [1] but no attempt has been made to elucidate the structure of the parent compound from which HCN is generated. One reason may have been the low cyanide levels which have been observed in most cyanogenic members of this family. Furthermore, some species are clearly polymorphic with regard to cyanogenesis (Hegnauer, R., personal communication) and in several tests cyanide could only be detected after more than 15–20 hr (Hegnauer, R., personal communication; Nahrstedt, A., unpublished results), a period which makes the test uncertain in normal cases. Hegnauer observed that aerial parts of *Sedum cepaea* L. growing in the southern Alps, as well as in Crete, show cyanogenesis when crushed. This material has been investigated and the structure of the main cyanogenic compound is presented here.

RESULTS AND DISCUSSION

The MeOH extract of the lyophilized aerial plant material, including stems and flowers, was chromatographed in successive steps on cellulose, polyamide and Si gel in order to remove most of the anthocyanins, sugars and organic acids. During all steps the substance was monitored for its cyanogenic properties in buffer (pH 6) with β-glucosidase added from sweet almonds, and by TLC. A final purification by HPLC on RP-18 yielded a white homogeneous, hygroscopic powder after lyophilization whose purity has been checked by HPLC, TLC and GC.

Hydrolysis with dilute H_3PO_4 yielded glucose (TLC, GC) and HCN; a carbonyl compound was not detectable. The compound was not stable in protic solvents and decomposed into glucose and HCN, while in MeCN no decomposition was observed. Using the pure substance it was noted, that β-glucosidase (from sweet almonds as well as from flax seeds) did not enhance the spontaneous HCN liberation in water. The MW of the compound is 291 (FDMS: $M^+ + 1 = 292$). This value is in agreement with the MW of the peracetate ($M^+ = m/z$ 501) and of the TMSi derivative ($M^+ = m/z$ 651) as five derivatizable OH groups were calculated from the ^{1}H NMR of the peracetate. From elemental analysis the C:N ratio was found to be 11:1.02; thus, the only nitrogen in the molecule is located in the nitrile group. The ^{1}H NMR spectrum of the underivatized compound showed a complex pattern of signals between 3.2 and 4.5 ppm indicating a non-aromatic substance with several —CH_x—OR groupings. The ^{13}C NMR spectrum showed 11 carbon resonances of which the glucose carbon resonances could be unambiguously assigned by reference to previous data [2] (Table 1). Additional resonances of a —CN group (116.78 ppm, *s*), a >CH—OR group (58.73 ppm, *d*), two —CH_2—OR groups (60.98, 67.83 ppm, *t*) and a quaternary carbon (53.92 ppm, *s*) were also present (Table 1). The present data, especially the rather high field resonance of the quaternary carbon at 53.92 ppm, suggest a butyronitrile derivative with an oxiran grouping, a —CH_2OR group and the nitrile group all attached to the quaternary carbon. Thus structures **1** and **2** are possible.

The 400 MHz ^{1}H spectrum of the pentaacetate was completely analysed (Table 2). The resonances of individual protons were assigned by homonuclear double resonance and the values of the shifts and couplings were calculated by a modified LACOON III program. The magnitude of the geminal coupling of 12.6 Hz of the isolated AB system (H5) clearly rules out a terminal epoxide, which should be *ca* (+)4 to (+)6 Hz [3], and thus structure **2a** and **2b** are eliminated. The coupling constant of 7.9 Hz of the C-1′ proton (4.60 ppm, *d*) confirms the β-configuration of the glucosidic linkage at C4 or C5 of **1a** or **1b**, respectively.

Alternative structures, **3**–**5**, of the aglycone moiety, incorporating 4- and 5-membered rings, were eliminated

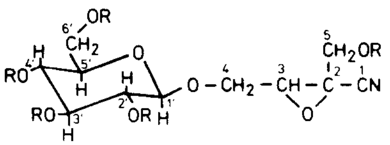
---

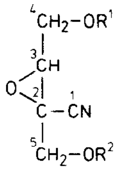
* Part of the projected dissertation of A. Walther.

Table 1. ^{13}C NMR data of the acetylated and underivatized compound **1b** in DMSO-d_6, as well as shift differences

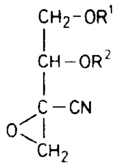
Carbon no.	R = H Shifts (ppm)	Multiplicity	R = CH$_3$CO Shifts (ppm)	Multiplicity	Δ (ppm) (acetate-underiv.)
1	116.78	*s*	115.44	*s*	−1.34
2	53.92	*s*	50.50	*s*	−3.32
3	58.73	*d*	58.92	*d*	+0.19
4	60.98†	*t*	61.61†	*t*	+0.63
5	67.83†	*t*	67.75†	*t*	−0.08
1′	103.60	*d*	99.81	*d*	−3.79
2′	73.35	*d*	70.78	*d*	−2.57
3′	77.02*	*d*	72.07*	*d*	−4.95
4′	69.91	*d*	68.08	*d*	−1.83
5′	76.63*	*d*	70.96*	*d*	−5.67
6′	61.72	*t*	63.49	*t*	+1.77
CH$_3$—$\underline{C}$O			170.15; 169.65; 169.37; 169.20;	*s*	
$\underline{C}$H$_3$—CO			20.51; 20.40 (×2) 20.31 (×2)	*q*	

* And † resonance assignments are interchangeable.





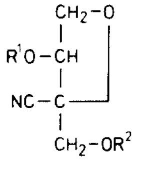
1a R^1 = H, R^2 = Glc
1b R^1 = Glc, R^2 = H
(sarmentosin epoxide)



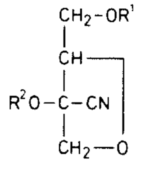
2a R^1 = H, R^2 = Glc
2b R^1 = Glc, R^2 = H

Table 2. ^{1}H NMR data for the pentaacetate of **1b** in CDCl$_3$

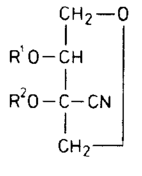
Proton	Shift (ppm)	Coupled protons	Coupling (Hz)
H3	3.43	(H3 H4A)	5.2
H4A	4.01	(H3 H4B)	5.4
H4B	3.99	(H4A H4B)	(−) 12.6
H5A	4.50	(H5A H5B)	(−) 12.6
H5B	4.13		
H1′	4.61	(H1′-H2′)	7.9
H2′	5.02	(H2′-H3′)	9.7
H3′	5.20	(H3′ H4′)	9.5
H4′	5.08	(H4′ H5′)	10.1
H5′	3.74	(H5′-H6′)	2.3
H6′	4.25	(H5′-H6″)	4.6
H6″	4.22	(H6′ H6″)	(−) 12.8
CH$_3$CO	2.15, 2.09, 2.06, 2.03, 2.00		



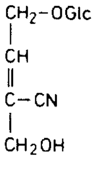
3a R^1 = H, R^2 = Glc
3b R^1 = Glc, R^2 = H



4a R^1 = H, R^2 = Glc
4b R^1 = Glc, R^2 = H



5a R^1 = H, R^2 = Glc
5b R^1 = Glc, R^2 = H



6 sarmentosin [11]

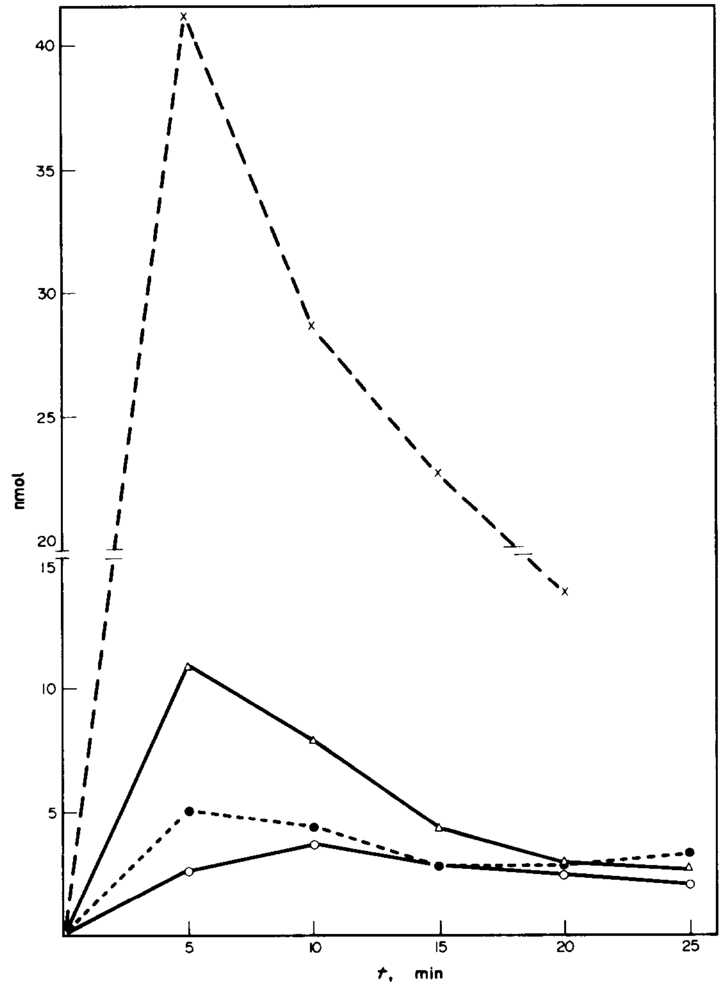


Fig. 1. HCN liberation from sarmentosin epoxide dissolved in buffer alone (● --- ●) and when rat liver microsomes have been added (△——△). A mixture of β-glucosidases (from sweet almonds and a crude preparation from flax seeds [13]) had no significant influence (○——○). The activity of the epoxide hydrases against the synthetic test substrate p-nitrostyrene epoxide under the same conditions is shown by × --- ×.

as the quaternary carbon resonance would be expected to be to lower field in such systems [4]. In addition, the magnitude of the geminal proton–proton coupling constant of the ring CH_2 group is *ca* (–)6 Hz for system **3** and **4** [5] and *ca* (–)9 Hz for system **5** [6], whereas a value of (–)12.6 Hz was found experimentally.

The position of the glucose moiety in **1** was deduced from the chemical shift differences in the ^{13}C NMR spectra of the acetylated and underivatized compound in DMSO-d_6 (Table 1). The shift difference of the glucose carbon resonances are in agreement with published data [7]. Acetylation is known to cause a large upfield shift of the β-carbon atom resonance of 3–5 ppm [8]. Thus the large upfield shift of the quaternary carbon, C2, and small shift of the C3 upon acetylation are only compatible with structure **1b**. Thus, the main cyanogenic compound of *S. cepaea* is 4-β-D-glucopyranosyloxy-2,3-epoxy-2-hydroxymethylbutyronitrile.

Proton nuclear Overhauser enhancement difference spectra at 400 Hz showed unambiguous enhancements for H3 and H5A upon irradiation of H5B, without any significant enhancement of H4; while irradiation of H3 caused enhancements of H4 and H5B and a smaller enhancement of H5A. Thus the configuration at C2 and C3 in **1b** is *RR* or *SS* with the CH_2O groupings *trans* to one another.

The new compound is not a typical cyanogenic glucoside as the cyanohydrin structure is lacking. However, acidic or alkaline cleavage of the oxiran ring is known for several epoxides [9]. In the case of the present compound a free cyanohydrin obviously results after hydrolysis of the oxiran group in water which then liberates HCN spontaneously as observed for the pure compound (see above). Consequently, a crude preparation of microsomes from rat liver containing epoxide hydrases [10] doubled the rate of HCN liberation as shown in the figure, whereas β-glucosidases had no significant effect on the rate of cyanogenesis. The new compound is the epoxide of sarmentosin (**6**) which was reported during the course of our work by a Chinese group as an antihepatotoxic compound from *S. sarmentosum* [11]. Although no information on the biogenetic origin of either glucoside is yet available, the carbon skeleton of **1b** and **6** suggests isoleucine as the precursor in a biogenetic pathway by analogy with those found for the cyanogenic glycosides [12].

EXPERIMENTAL

Plant material was collected in the southern Alps of Switzerland (Ticino) and kindly provided by Prof. Hegnauer (Leiden). The plants were propagated in the Botanical Garden of the Technical University of Braunschweig. The aerial plant material was collected during the flowering stage, immediately frozen under liquid N_2 and ground under liquid N_2. After lyophilization it was stored at −18°.

Extraction and purification. 150 g dry material was extrd with petrol and the extract discarded. The residue was extrd in cold MeOH using an Ultra Turrax and the extract concd and chromatographed on cellulose (Avicel, Merck, 100 × 4.2 cm) with *iso*-PrOH–*n*BuOH–H_2O (7:2:1). Cyanogenesis was observed within the fraction from 1200 to 1900 ml. This was concd and chromatographed on a polyamide column (65 × 3 cm) with H_2O as the solvent. The lyophilized cyanogenic fraction (150–300 ml) was then chromatographed on Si gel (70 × 3 cm) with EtOAc–MeOH–HOAc (50%) (90:10:0.04). The concd cyanogenic fractions (620–1000 ml) were finally purified by HPLC on Lichrosorb RP-18. The cyanogenic fractions were dried by lyophilization and the resulting white, hygroscopic powder used for further investigation.

Elemental analysis. Found 43.66% (C), 6.07% (H), 4.72% (N), 45.55% (O, diff.). Calc.: 45.36% (C), 5.84% (H), 4.81% (N), 43.98% (O).

Hydrolysis. (a) 1 N H_3PO_4, 1 hr, 100°. (b) 1 mg/ml β-glucosidase (Serva, Heidelberg) and/or linamarase [10] in 0.1 M citrate buffer pH 6, 38°. (c) Water, doubly distilled, 38°.

HCN *estimation.* The Feigl–Anger test [14] was used for qualitative analysis and for monitoring the column eluates. The pyridine 2-ABA method [15] was used for quantitative estimations.

Derivatization. The TMSi–ether was prepared as described in [16] in the presence of pyridine. The peracetate was prepared by dissolving 30 mg of the pure compound in 3 ml of pyridine–Ac_2O (1:1) for 24 hr. The solvent was then evapd at 0.2 torr and the residue used for analysis.

TLC. Sarmentosin epoxide: Si gel/EtOAc–MeOH–H_2O–HOAc (80:20:1:1); R_f: 0.45. Detection with anisaldehyde spray reagent (intact glucoside) and the sandwich technique (HCN) as described in [14]. Hexoses by [17].

GC. Sarmentosin epoxide as TMSi-ether on OV-225, 3% on Gaschrom Q 100 120 mesh, 2.5 m × 2 mm i.d., steel; 150- 230°, 2°/min. N_2 25 ml/min. R_t: 41.7 min. Hexoses after reduction to the corresponding cyclitols and acetylation on ECNSS-M 3% on Gaschrom Q 100 120 mesh, steel, 165 205°, 4°/min, N_2 25 ml/min.

HPLC. (Analytical) Lichrosorb RP-18, 10 μm, 0.4 × 25 cm; H_2O–MeOH–HOAc 97.5:2.5:0.2, 1.25 ml/min, RI detector R_t: 6.6 min. (Prep.): Lichrosorb RP-18, 10 μm, 1.6 × 25 cm. H_2O–MeOH–HOAc 97.5:2.5:0.1. RI detector. 5.25 ml/min. R_t: 16.2 min.

NMR. Spectra were recorded at ambient temp. on either a Varian XL-100-12 (^1H and ^{13}C) and a Bruker WM 400 (^1H) spectrometer operating in the Fourier transform mode and locked to the deuterium resonance of the solvent. Shifts are reported in ppm relative to TMS. Nuclear Overhauser enhancements ^1H difference spectra were recorded on the Bruker instrument using the standard software package.

Hydrolysis by liver microsomes. Livers of two Sprague–Dawley rats were dissected and the microsomes fraction prepared by ultracentrifugation [18]. The activity of the epoxide hydrases was tested with *p*-nitrostyrene epoxide as described in [18] using an HPLC system (Lichrosorb) RP-18 0.4 × 25 cm, H_2O – MeOH (3:2), 2 ml/min, detection UV 254 nm). In order to estimate the kinetics of cyanide liberation from sarmentosin epoxide by the liver microsomes in KPi buffer (0.1 M, pH 7, 38°) the apparatus developed by Wissing [19] was used and the HCN estimated by the pyridine–2-ABA method [15]. Incubation was carried out with 100 μl of the microsome prepn (765 μg protein), 800 μl buffer and 2.14 μmol sarmentosin epoxide in 75 μl MeCN. The activity of the epoxide hydrases was tested with 0.1 ml microsome prepn, 475 μl buffer and 0.46 μmol *p*-nitrostyrene epoxide in 45 μl MeCN. Under these conditions MeCN alone did not liberate measurable amounts of HCN.

Acknowledgements We are most grateful to Professor R. Hegnauer (Leiden) who provided us with the living plant material. We thank Dr. M. Schiebel, Organisch-chemisches Institut der TU Braunschweig, who performed the EIMS spectra, Dr. Spitzner, Universität Stuttgart-Hohenheim, who performed the FDMS, Professor Schüppel and J. Böttcher, Pharmakologisches Institut der TU Braunschweig, who kindly helped during the preparation of the microsome fraction from rat livers and Dr. D. Geffken, Institut für Pharmazeutische Chemie der TU Braunschweig, who carried out the elemental analysis.

REFERENCES

1. Hegnauer, R. (1964) *Chemotaxonomie der Pflanzen*, Vol. 3, p. 583. Birkhäuser Verlag, Basel.
2. Hübel, W., Nahrstedt, A. and Wray, V. (1981) *Arch. Pharm.* **314**, 609.
3. Jackmann, L. M. and Sternhell, S. (1969) *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* 2nd edn, p. 272. Pergamon Press, Oxford.
4. Bremser, W., Ernst, L., Franke, B., Gerhards, R. and Hardt, A. (1979) *Carbon-13 NMR Spectral Data*. Verlag Chemie, Weinheim.
5. Batterham, T. J. (1972) *NMR Spectra of Simple Heterocycles*, p. 369. John Wiley, New York.
6. Botteghi, C., Consiglio, G., Ceccarelli, G. and Stefani, A. (1972) *J. Ord. Chem.* **37**, 1835.
7. Seo, S., Tomita, Y., Tori, K. and Yoshimura, Y. (1978) *J. Am. Chem. Soc.* **100**, 3331.
8. Stothers, J. B. (1972) *Carbon-13 NMR Spectroscopy*, p. 150. Academic Press, New York.
9. Buchanan, J. G. and Sable, H. Z. (1972) in *Selective Organic Transformations* (Thyagarajan, B. S., ed.) Vol. 2, p. 1. John Wiley–Interscience, New York.
10. Oesch, F. (1972) *Xenobiotica* **3**, 305.
11. Fang shen-diu, Yan Xin-quan, Li Ching-fang, Fan Zhi-yun, Xy Xia-yi and Xu Jen-sheng (1979) *Kexue Tongbao* **24**, 431.
12. Conn, E. E. (1979) *Naturwissenschaften* **66**, 28.
13. Coop, I. E. (1940) *N. Z. J. Sci. Technol.* **22**, 71B.
14. Tantisewie, B., Riujgrok, H. W. L. and Hegnauer, R. (1969) *Pharm. Weekblad* **104**, 1341.
15. Nahrstedt, A. (1977) *Dtsch. Apoth. Z.* **117**, 1357.
16. Klebe, J. F., Finkbeiner, H. and White, D. M. (1966). *J. Am. Chem. Soc.* **88**, 3390.
17. Wiehle, H. and Horak, E. (1970) *J. Chromatogr.* **47**, 527.
18. Westkaemper, R. B. and Hanzlik, R. P. (1980) *Analyt. Biochem.* **102**, 63.
19. Wissing, F. (1973) *J. Bacteriol.* **117**, 1289.