Phytochemistry, Vol. 21, No. 1, pp. 101–105, 1982.
Printed in Great Britain.

0031–9422/82/010101–05 $03.00/0

ACALYPHIN, A CYANOGENIC GLUCOSIDE FROM *ACALYPHA INDICA**

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(*Received* 5 *May* 1981)

Key Word Index—*Acalypha indica*; Euphorbiaceae; Acalypheae; cyanogenic glucoside; acalyphin; 3-cyano-3-β-D-glucopyranosyloxy-2-hydroxy-4-methoxy-1-methyl-6(2,3-dihydro)pyridone.

Abstract—A new cyanogenic glucoside, acalyphin, was isolated from the aerial parts of *Acalypha indica* and its structure identified mainly by ^{1}H NMR and ^{13}C NMR methods as 3-cyano-3-β-D-glucopyranosyloxy-2-hydroxy-4-methoxy-1-methyl-6(2,3-dihydro)pyridone. The new compound represents a new biogenetic type of cyanogenic glycoside.

INTRODUCTION

Acalypha indica L. is an annual weed growing in the Indian peninsula, the southern districts of China, in Abyssinia, South Africa, Mexico and Arizona [1–3]. The aerial parts and roots are used in folk medicine [4], and the plant has been described in homoeopathic pharmacopoeias [5, 6]. Cyanogenesis of *Acalypha indica* has been known for a long time [2]. In 1937 Rimington and Roets [7] investigated the cyanogenic principle assuming that this substance caused loss of livestock which had grazed on *A. indica*, as the symptoms were similar to cyanide intoxication [8]. A substance with mp 182–184° was isolated and the elemental composition was found to be $C_{14}H_{20-22}N_2O_{10}$, but the structure was not established [7]. In the present paper the structure of the cyanogenic compound of *A. indica* is presented.

RESULTS AND DISCUSSION

The air-dried strongly cyanogenic aerial parts of *A. indica* were powdered and extracted with cold MeOH; the solvent was evaporated and the residue suspended in water. Lipophilic components were eliminated by extraction with CH_2Cl_2. The water phase was chromatographed on polyamide and cellulose and finally purified by HPLC on RP-18. The cyanogenic compound was monitored both by TLC using UV detection, and by making use of its cyanogenic properties produced by the action of linamarase [9]. After lyophilization a colourless hygroscopic substance was produced whose purity was checked by HPLC, GLC and TLC. Contrary to statements made previously [7, 10, 19], the purified substance is stable in aqueous solution for several weeks. Although hydrolysis with β-glucosidase from sweet almonds does not occur, the weak reaction with linamarase [9] yields glucose (TLC, GLC) and HCN (Feigl–Anger test [11]); a carbonyl compound was not detectable. On the other hand, plant material incubated with water is strongly cyanogenic, indicating that a selective β-glucosidase is present in this material.

The UV spectrum showed an absorption at 223 nm (log ε 3.80) and a shoulder at 255 nm (log ε 3.39). The IR spectrum indicated a tertiary amide grouping from absorptions at 1630 and 1670 cm^{-1}. A nitrile absorption was not visible, so that an α,β-saturated cyanohydrin structure was expected [12]. The MW was estimated by FDMS as 360 (M + 1 = 361). This value was confirmed by the EIMS of the peracetylated compound ($M^+ = m/z$ 570) as five derivatizable hydroxyl groups were estimated by the ^{1}H NMR of the peracetate (see Table 1). From the elemental analysis a C:N ratio was calculated as 14:1.8 indicating that the substance contains one more nitrogen than that located in the CN group.

On the basis of 14 carbon atoms, the elemental analysis and the data above a molecular formula of $C_{14}H_{20}N_2O_9$ was derived. This, together with the observed mp of the underivatized compound of 185–186°, indicate that the present substance is identical with that formerly isolated by Rimington and Roets [7].

The ^{1}H NMR spectral data for the underivatized compound in several solvents and the pentaacetate in $CDCl_3$ are given in Table 1. For the former the H1′ resonance of glucose occurs at 4.7 ppm with a J(H1′–H2′) value of 7.8 Hz indicative of a β-glucosidic linkage. A resonance of an *N*-Me group was observed at about 3 ppm and that of an *O*-Me group at 3.8 ppm. Two further singlets occurred in the range 5.2–5.4 ppm: one methine proton at 5.3 ppm and another bound to a secondary alcoholic function which is sharp in D_2O but broad in aprotic solvents. In the latter solvents addition of H_2O causes the broad signal to split into a doublet with a characteristic vicinal =CH–OH coupling of 5 Hz [13]. Nuclear Overhauser enhancement ^{1}H difference spectra at 400 MHz of the pentaacetate showed unambiguous enhancements of the resonance at 5.4 ppm upon irradiation of the *O*-methyl singlet and at 6.4 ppm upon irradiation of the *N*-methyl singlet, and vice versa. This clearly suggests a vicinal arrangement of the respective groups.

* Part of the projected dissertation of J.-D. Kant.

Table 1. ^{1}H NMR data for acalyphin, **1a** and its pentaacetate, **1b** (shifts in ppm relative to TMS)

	Shifts				
	1a			**1b**	
Solvent	D_2O	MeCN-d_3	Acetone-d_6	$CDCl_3$	Multiplicity*
N-C<u>H</u>$_3$	3.01	2.95	2.96	3.00	*s*
O-C<u>H</u>$_3$	3.86	3.81	3.81	3.84	*s*
	5.3	5.29	5.27	5.76	*s*
	5.43	5.21 (broad)	5.34 (broad)	6.37	*s*
H1′	4.73	4.67	4.73	3.97–5.31	*d*†
H2′–H6′	3–3.8	3–3.8	3–3.8		
C<u>H</u>$_3$CO				1.98; 1.99; 2.03; 2.11; 2.13	*s*; *s*; *s*; *s*; *s*;

* *s* = singlet and *d* = doublet.
† ^3J(H1′–H2′) 7.8 Hz.

The ^{13}C NMR spectrum of the underivatized compound in DMSO-d_6 showed 14 C atoms of which the glucose C resonances could be unambiguously assigned by reference to previous data [14] (Table 2). In addition, the carbon resonances of a nitrile group (114.83, *s*), a *N*-methyl group (31.62, *q*) an *O*-methyl group (56.97, *q*) a =CH– group (96.82, *d*), a =C(OCH$_3$)– (158.75, *s*) and two further quaternary carbon atoms at 77.78 and 163.08 ppm were observed. Of the latter two resonances the high-field one is consistent with a =C(CN)–O– Glc grouping and the low-field one with a –CO–N(Me)– grouping. Table 2 also shows the ^{13}C NMR data of the peracetylated compound as well as the shift differences between this and the underivatized substance. The shift differences of the glucose C resonances are in agreement with published data [15]. Acetylation is known to cause a large upfield shift of the β-carbon atom of 3–5 ppm [16]. Thus, the large upfield shift of the quaternary carbon C3 of the cyanohydrin grouping indicates that it is vicinal to the =CH–OAc group in the molecule.

The present data are only consistent with either structure **1** or **2**. The multiplicities of the resonances in the proton-coupled C spectrum of the pentaacetate (Table 2) enable these to be distinguished. The magnitude of the long-range coupling ^3J(CH) is known to be significant and greater than ^4J(CH) [17]. Thus the olefinic carbon resonance (C5 of **1** and C4 of **2**) would appear as a doublet for **1** [^1J(CH) only] and as a doublet of doublets for **2** [^1J(CH) and ^3J(CH2)]. Similarly, the resonance of the nitrile carbon would appear as a doublet for **1** [^3J(CH2)] and a doublet of doublets for **2** [^3J(CH2) and ^3J(CH4)] and the resonance of carbon 2 would appear as a doublet of quartets for **1** [^1J(CH2) and ^3J(CH7)] and a doublet of doublets of quartets for **2** [^1J(CH2), ^3J(CH7) and ^3J(CH4)]. Only **1** is compatible with the experimental data (Table 2).

Thus the cyanogenic compound of *A. indica*, acalyphin, is 3-cyano-3-β-D-glucopyranosyloxy-2-hydroxy-4-methoxy-1-methyl-6(2,3-dihydro)pyridone (**1a**). The configuration at C2 and C3, however, has not been established.

Acalyphin is hydrolysed by strong acids (27–35% HCl) yielding glucose and a second component with a M^+ at *m/z* 155 and after addition of D_2O a M^+ at *m/z* 156 indicating one free OH group. The ^{1}H NMR of this compound in D_2O showed a *N*-methyl at 3.43 ppm and an *O*-methyl at 3.86 ppm and two further protons at 6.04 and 7.17 ppm. Nuclear Overhauser enhancement ^{1}H difference spectra showed enhancement of the 6.04 ppm signal upon irradiation of the OMe group. A smaller enhancement of the 7.17 ppm signal was observed upon irradiation of the *N*-methyl group. From the reaction behaviour of the cyanogenic glycosides, hydrolysis to α-hydroxy acids by strong acids is well known [12]. Thus, acalyphin is hydrolysed to the corresponding α-hydroxy acid which decarboxylates spontaneously and then eliminates one molecule of water producing 3-hydroxy-4-methoxy-*N*-methyl-6-pyridone (HMMD).

The new compound is named acalyphin as this has been used previously to describe the cyanogenic principle of *A. indica* [18, 19]. However, this name was at first provisionally proposed for an unknown 'alkaloidal' substance contained in an ether extract from *A. indica* leaves dried at low temperature [20], but the 'alkaloidal principle' was never established. Triacetonamine which reacts positively with alkaloid reagents was demonstrated to be a constituent of *A. indica* extracts, but obviously is an artefact of the isolation procedure [21].

Acalyphin is a cyanopyridone derivative and is thus similar in structure to ricinine [22], ricinidine [23], nudiflorine [24], and mallorepine [25] all of which have been isolated from the tribe Acalypheae of the subfamily Crotonoideae within the Euphorbiaceae. Thus, the Acalypheae, with respect to their cyanogenic glycosides, clearly differ from other cyanogenic tribes of the Crotonoideae which have been shown to contain linamarin and lotaustralin [10]. Although no information of the biogenetic precursor is yet available, a similar pathway must be postulated to that suggested for the 3-cyanopyridones mentioned above, which is closely connected with the NAD cycle [22]. Thus, acalyphin represents a new biogenetic type of cyanogenic glycoside

Table 2. ^{13}C NMR data for acalyphin, **1a** and its pentaacetate, **1b** (shifts in ppm relative to TMS)

	1a (DMSO-d_6)*		**1b** (DMSO-d_6)*			**1b** ($CDCl_3$)‖		
Carbon no.	Shift (ppm)	Multiplicity† in SFORD‡ spectrum	Shift (ppm)	Multiplicity† in SFORD‡ spectrum	Δ(**1b** − **1a**) (ppm)	Shift (ppm)	Multiplicity† in ^1H coupled spectrum	J(CH) (Hz)
2	82.89	*d*	81.42	*d*	−0.87	81.90	*d*, *q*	168.8, 4.3
3	77.78	*s*	74.03	*s*	−3.75	74.85	*d*, *d*, *d*	8.6, 4.4, 4.4
4	158.75	*s*	157.99	*s*	−0.76	158.39	*m*	3.7
5	96.82	*d*	96.96	*d*	+0.14	97.50	*d*	169.1
6	163.08	*s*	162.85	*s*	−0.23	163.27	*d*, *q*	6.1, 3.1
7	31.62	*q*	32.67	*q*	+1.05	33.27	*q*, *d*	140.4, 3.1
8	56.97	*q*	57.82	*q*	+0.85	57.13	*q*	147.3
9	114.83	*s*	113.22	*s*	−1.61	112.42	*d*	1.2
1′	100.70	*d*	97.32	*d*	−3.38	98.43	*d*, *d*, *d*	163.3, 7.0, 2.8
2′	72.80	*d*	70.25	*d*	−2.55	70.49	*d*, *d*	153.8, 4.3
3′	77.59§	*d*	71.24	*d*	−6.35	72.71	*d*, *b*	144.7
4′	69.66	*d*	68.12	*d*	−1.54	68.13	*d*, *b*	150.8
5′	76.66§	*d*	71.24	*d*	−5.42	72.15	*d*, *b*¶	148.9
6′	60.95	*t*	61.84	*t*	+0.87	61.82	*t*, *b*	149.2
C̲H$_3$CO			20.33; 20.24; 20.01	*q*		20.57, 20.48 (×3); 20.29	*q*	129.8
MeC̲O			170.03; 169.49; 169.31 (×2); 168.70	*s*		170.57	*q*, *t*	7.3, 3.4
						169.92; 169.21	*q*, *d* (each)	7.3, 3.7 (each)
						169.26; 168.85	*q*, *d* (each)	6.7, 3.7 (each)

* Recorded on Varian XL-100-12 spectrometer.

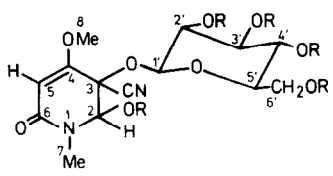
† *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet and *b* = broad.

‡ Proton single frequency off-resonance decoupled spectrum.

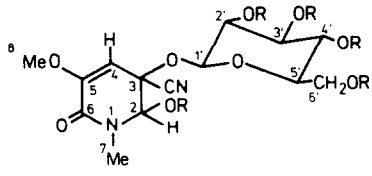
§ Interchangeable.

‖ Recorded on Bruker WM 400 spectrometer.

¶ Broader than resonance for C3′.



1a R = H
1b R = Ac



2a R = H
2b R = Ac

EXPERIMENTAL

Plant material. The aerial parts of *A. indica*, including flowers, have been collected by members of the staff of the Homoeopathic Pharmacopoeia Laboratory (Government of India, Ghaziabad, India) and carefully air-dried. The material was ground for extraction.

Extraction and purification. Powdered material (20 g) was extracted with cold MeOH using an Ultra Turrax (Jahnke & Kunkel, D-Staufen). The MeOH extract was concd under vac. to dryness, the residue suspended in water, filtered and extracted with CH_2Cl_2. The aq. phase was lyophilized, the residue was dissolved in 25 ml H_2O and filtered through a polyamide column (1.4 × 14 cm). The eluate was lyophilized again and chromatographed on cellulose (2.5 × 70 cm) with *n*-BuOH satd with water; the cyanogenic substance was eluted in 390–520 ml. The residue of the lyophilized positive fractions was dissolved in 1 ml water and finally purified by HPLC on Lichrosorb RP-18 (7 μm, 1.6 × 25 cm, MeCN H_2O 4:96, 3.5 ml/min, detection 250 nm, t_R 20.4 min). The lyophilized fractions resulted in 60 mg colourless hygroscopic powder which was used for further investigation. TLC systems for acalyphin: Me_2CO–MeOH H_2O, 5:1:0.5, R_f 0.23; MeCOEt EtOAc–MeOH–H_2O, 5:3:1:1, R_f 0.60. GLC system for acalyphin TMSi ether: OV-101, 1.5% on Chromosorb W, HP 80–100 mesh, 1.8 m × 0.2 mm i.d., 210–260°, 1°/min, nitrogen 25 ml/min, FID, t_R 30 min.

Hydrolysis. (a) With crude linamarase [9] 2 mg/ml in Pi buffer, pH 5.5, at room temp. (hydrolysis product measured: HCN). (b) With 0.1 N HCl, 2 hr, 100° (product found: HCN and glucose). (c) With 25% or 37% HCl, 1 hr, 100° (product found: glucose and 3-hydroxy-4-methoxy-1-methyl-6-pyridone = HMMP).

HCN estimation. Feigl–Anger test [11] for qualitative analysis and for monitoring the column eluates using crude linamarase. Quantitative by the pyridine 2-ABA method [27].

Glucose estimation. By TLC [26] and after reduction to the corresponding polyol and acetylation by GLC on ECNSS-M, 3% on Gaschrom Q 100–120 mesh, 1.80 m × 2 mm i.d. steel; 190 isothermally. t_R of sorbitol acetate: 42 min.

HMMP. The hydrolysis mixture was extrd with Et_2O and the Et_2O phase discarded. The aq. phase was lyophilized and purified by TLC (Si gel EtOAc MeOH–H_2O–AcOH, 70:20:5:5; R_f glucose 0.32; R_f HMMP 0.56; R_f acalyphin 0.51). The glucose zone was sepd and used for further investigation. The HMMP zone was also sepd and used for ^{1}H NMR and EIMS.

Derivatization. The peracetate was prepared by leaving 10 mg acalyphin in 0.5 ml pyridine and 0.5 ml Ac_2O for 24 hr. The solvents were then evapd, the residue was dissolved in a small amount of MeOH, water was added, the precipitate sepd by centrifugation and washed with cold water.

NMR spectroscopy. The spectra have been recorded at room temp. on either a Varian XL-100-12 or a Bruker WM 400 spectrometer operating in the Fourier transform mode and locked to the deuterium resonance of the solvent. Shifts are reported relative to TMS. Nuclear Overhauser enhancement ^{1}H difference spectra were recorded on the Bruker instrument using the standard software package.

Acknowledgements—We thank Dr. P. N. Varma (Homoeopathic Pharmacopoeia Laboratory, Government of India, Ghaziabad, India) who supplied us with the plant material. We thank Dr. L. Witte (GBF, Braunschweig-Stöckheim) who performed the EIMS, Dr. Spitzner (Univ. Stuttgart-Hohenheim) who performed the FDMS, and Dr. D. Geffken (Techn. Univ. Braunschweig) who carried out the elemental analysis.

REFERENCES

1. (1969) *Hagers Handbuch der Pharmazeutischen Praxis* (Kern, W., List, P. H. and Hörhammer, L., eds.) 4th edn, p. 876. Springer Verlag, Heidelberg.
2. Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa* 2nd edn, p. 394. Livingstone, Edinburgh.
3. Pax, F. and Hoffmann, K. (1931) in *Die natürlichen Pflanzenfamilien* (Engler, A. and Harms, H., eds.) 2nd edn, Vol. 19c, p. 135. Verlag Wilhelm Engelmann, Leipzig.
4. Madaus, G. (1938) *Lehrbuch der biologischen Heilmittel*. Vol. 1, p. 380. Georg Thieme Verlag, Leipzig.
5. (1941) *The Homoeopathic Pharmacopoeia of the United States* 6th edn, p. 53. Otis Clapp, Boston.
6. (1971) *Homoeopathic Pharmacopoeia of India* 1st edn, Vol. 1, p. 33. Controller of Publications, Delhi.
7. Rimington, C. and Roets, G. C. S. (1937) *Onderstepoort J. Vet. Sci. Animal Ind.* **9**, 193.
8. Steyn, D. G. (1937) *Onderstepoort J. Vet. Sci. Animal Ind.* **9**, 573.
9. Coop, I. E. (1940) *N.Z. J. Sci. Technol.* **22**, 71B.
10. van Valen, F. (1978) *Planta Med.* **34**, 408.
11. Tantisewie, B., Ruijgrok, H. W. L. and Hegnauer, R. (1969) *Pharm. Weekblad* **104**, 1341.
12. Nahrstedt, A. (1981) in *Cyanide in Biology* (Vennesland, B., Conn, E. E., Knowles, C. J., Westley, I. and Wissing, F., eds.). Academic Press, London.
13. Jackman, L. M. and Sternhell, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd edn, p. 298. Pergamon Press, Oxford.
14. Hübel, W., Nahrstedt, A. and Wray, V. (1981) *Arch. Pharm.* **314**, 609
15. Seo, S., Tomita, Y., Tori, K. and Yoshimura, Y. (1978) *J. Am. Chem. Soc.* **100**, 3331.
16. Stothers, J. B. (1972) *Carbon-13 NMR Spectroscopy*, p. 150. Academic Press, New York.

17. Hansen, P. E. (1981) *Progr. Nucl. Magn. Reson. Spectros.* (in press).
18. Eyjólfsson, R. (1970) *Fortsch. Chem. Org. Naturst.* **28**, 74.
19. Hegnauer, R. (1977) *Plant Syst. Evol. Suppl.* **1**, 191.
20. Dymock, W., Warden, C. J. H. and Hooper, D. (1890) *Pharmacographia Indica, a History of the Principal Drugs of Vegetable Origin*, Part 3, p. 294. Kegan Paul, Trench, Trüher, London.
21. Martinez, S. and Ruiz, E. (1980) *Rev. Cub. Farm.* **14**, 29.
22. Waller, R. and Nowacki, E. K. (1978) *Alkaloid Biology and Metabolism in Plants*. Plenum Press, New York.
23. Gauguly, S. N. (1970) *Phytochemistry* **9**, 1667.
24. Mukherjee, R. and Chatterjee, A. (1966) *Tetrahedron* **22**, 1461.
25. Hikino, H., Tamada, M. and Kun-Ying Yen (1978) *Planta Med.* **33**, 385.
26. Wiehle, H. and Horak, E. (1970) *J. Chromatogr.* **47**, 527.
27. Nahrstedt, A. (1977) *Dtsch. Apoth. Z.* **117**, 1357.