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THE STRUCTURES OF TETRAPHYLLIN A AND B, TWO NEW CYANOGLUCOSIDES FROM *TETRAPATHAEA TETRANDRA*

G. B. RUSSELL and P. F. REAY

Applied Biochemistry Division, D S I R, Palmerston North, New Zealand

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Abstract—Two new cyanoglucosides, tetraphyllin A and B (*O*-β-D-glucosyl-1-cyano-1-hydroxy-2-cyclopentene and 1-*O*-β-D-glucosyl-1-cyano-1,4-dihydroxy-2-cyclopentene respectively) were isolated from the fruit of *Tetrapathaea tetrandra* and their structures elucidated.

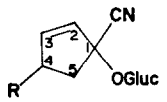
INTRODUCTION

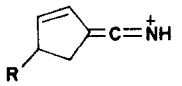
SEVERAL members of the Passifloraceae have been reported to be cyanogenic;[1,2] recently Tantisewie *et al.*[3] examined several species of this family and concluded that the major glucoside is probably gynocardin. Paris *et al.*[4] have isolated from *Baheria fistulosa* a new crystalline cyanoglucoside which they concluded was a derivative of cyclopentene.

In studying the New Zealand passion fruit *Tetrapathaea tetrandra* we noted evolution of HCN from the cold ethanol extracts and we have isolated two crystalline cyanogenic glucosides from the immature fruit in moderate yields. The structures (I) and (II) have been determined from spectroscopic evidence derived from the compounds and their derivatives. Tetraphyllin A and B are the trivial names proposed for these compounds, both of which contain a cyclopentenyl moiety. The stereochemistry of tetraphyllin B is under investigation.

RESULTS

Crystalline tetraphyllin A and B were isolated in yields of 0·06 and 0·4% respectively with a recovery of 24% of the cyanide present in the fruit (70 μmoles/g fr. wt.)





Tetraphyllin A, I R = H
Tetraphyllin B, II R = OH

III R = H
IV R = OH

Tetraphyllin A (I) had an empirical formula $C_{12}H_{17}NO_6$ determined from elemental analysis. Its mass spectrum showed peaks at *m/e* 222, $C_{11}H_{12}NO_4$ (M^+—49); *m/e* 92, C_6H_6N, representing the aglycone ion formed after loss of a glucosyloxy radical; *m/e* 93, C_6H_7N, consistent with an ion III arising from hydrogen transfer and elimination. It gave an acetate derivative whose NMR spectrum integrated for four acetyl groups.

[1] R. HEGNAUER, *Pharm Weekblad* **94**, 248 (1959)
[2] G. DILLEMAN, *Enc. Plant Phys* **8**, 1050 (1958).
[3] B TANTISEWIE, H. W L. RUIJGROK and R HEGNAUER, *Pharm. Weekblad.* **104**, 1341 (1969).
[4] M PARIS, A BOUQUET and R. PARIS, *C.R. Acad. Sci, Paris.* **268**, 2804-Ser. D (1969)

Tetraphyllin B (II) had an empirical formula $C_{12}H_{17}NO_7$ determined both by elemental analysis and by mass measurement of the molecular ion at *m/e* 287. The mass spectrum also gave ions at *m/e* 108, C_6H_6NO, produced by loss of a glucosyloxy radical and at *m/e* 109, C_6H_7NO, consistent with IV. It gave an acetate derivative whose NMR spectrum integrated for five acetyl groups.

Treatment of both tetraphyllin A and B with linamarase released HCN. For tetraphyllin A the recovery was only 47 per cent of the theoretical, while that for B was 96 per cent.

Acidic hydrolysis of tetraphyllin A gave an oil in good yield, whose mass spectrum showed a molecular ion at *m/e* 182, $C_{12}H_{10}N_2$ and whose i.r. spectrum showed nitrile absorption at ν 2290 cm^{-1}. These results suggest that the compound is probably a dicyanodicyclopentadiene adduct formed from dehydration and addition of the cyanohydrin intermediate. The NMR spectrum is consistent with the formation of such an adduct. Glucose was also released by the acid hydrolysis.

Acidic hydrolysis of tetraphyllin B gave the aglycone, 4-hydroxy-2-cyclopenten-1-one along with glucose. The u.v. maximum of the aglycone at 213 nm is consistent with the 2-cyclopentenone chromophore.[5]

Alkaline hydrolysis of tetraphyllin B with $Ba(OH)_2$ gave a carboxylic acid which was methylated and hydrolysed with linamarase to give methyl-1,4-dihydroxy-2-cyclopenten-1-carboxylate. Alkaline hydrolysis of tetraphyllin A followed by acidic hydrolysis gave an adduct whose NMR spectrum was similar to that adduct formed after acidic hydrolysis of tetraphyllin A.

The NMR spectra of the two glucosides contained signals at δ 4·73, (1H, d, $J = 7$ Hz) 3·80 (2H, m) and 3·45 (4H, m) which are consistent with a β-glucoside structure.[6] The spectrum of tetraphyllin B also showed pairs of doublets centered at δ 6·23 (1H), and 6·46 (1H), which are attributed to vinylic protons; their coupling constant (Table 1) indicates an unsymmetrically substituted five membered ring.[7] A multiplet at δ 5·07 (1H) arises from the proton on the oxygen bearing carbon (C-4); two further pairs of doublets, one centred at δ 2·23 (1H) and the other at 2·90 (1H) can be assigned to the nonequivalent geminal protons (C-5) of the cyclopentene ring. The spectrum of tetraphyllin A showed a broad singlet at δ 2·58 (4H), and doublets at δ 6·03 (1H) and 6·51 (1H); both of the latter signals are split by additional coupling. The cyclopentenyl moiety proposed for tetraphyllin A is in accord with these results.

Since the signal for the proton on the oxygen bearing carbon (C-4) remains at δ 4·91–5·07 for the three compounds, tetraphyllin B, the hydroxycyclopentenone and the ester derivative, and since the geminal coupling for the three compounds varies with the alteration of the C-1 substituent, the geminal protons are assigned to C-5 and the hydroxyl group is positioned on C-4 The splitting patterns observed for the vinylic protons support this assignment.

The structure of tetraphyllin A was confirmed with the conversion of its tetraacetate into a pentaacetate by treatment with *N*-bromosuccinimide followed by substitution of the bromine with acetate. The pentaacetate had the same mobility as tetraphyllin B-pentaacetate on TLC and GC and the NMR spectra of these two pentaacetates were identical.

Tetraphyllin A and B are closely related structurally to the cyanogenic glucoside

[5] W. M SCHUBERT and W. A SWEENEY, *J Am Chem Soc* **77**, 2297 (1955)
[6] J M VAN DER VEEN, *J Org Chem* **28**, 564 (1963)
[7] N S BHACCA and D H WILLIAMS, *Applications of NMR Spectroscopy in Organic Chemistry*, p 54, Holden-Day, San Francisco (1964)

TABLE 1. NMR DATA FOR TETRAPHYLLIN A AND B AND DERIVATIVES

Compounds	H_2	H_3	H_4	C_4 and C_5-CH_2	C_5-CH_2
Tetraphyllin A*	6 51, dm, $J = 6$	6 03, dm, $J = 6$		2 58, m	
Tetraphyllin B*	6 46, dd, $J = 6$	6 23, dd, $J = 6$	5 07, m		2 33, dd, $J = 15$, $J' = 4$ 5
	$J' = 1$	$J' = 1$			2 90, dd, $J = 15$, $J' = 6$
Tetraphyllin A-tetraacetate†	6 44, dm, $J = 6$	5 95, dm, $J = 6$		2 55, m	
Tetraphyllin B-pentaacetate †	6 32, dd, $J = 6$	6·03, dd, $J = 6$	5·80, m		2·38, dd, $J = 15$, $J' = 4$ 5
	$J' = 1$	$J' = 1$			2 87, dd, $J = 15$, $J' = 6$
4-Hydroxy-2-cyclopentenone†	7 51, dd, $J = 6$	6 15, dd, $J = 6$	4·98, m		2·15, dd, $J = 18$, $J' = 2$ 7
	$J' = 3$	$J' = 1$ 5			2·73, dd, $J = 18$, $J' = 6$
Tetraphyllinic acid B methyl ester*	6 35, dd, $J = 6$	6 19, dd, $J = 6$	5 08, m		2 01, dd, $J = 15$, $J' = 4$ 5
	$J' = 1·5$	$J' = 1$			2 68, dd, $J = 15$, $J' = 6$
Methyl-1,4-dihydroxy-2-cyclo-pentene-1-carboxylate†	5 73, d, $J = 6$	6 10, dd, $J = 6$	4 91, m		2 25, d, $J = 1·5$
		$J' = 2$			2 18, s

* Measured in D_2O.

† Measured in $CDCl_3$ at 60 MHz. Chemical shifts are given in ppm (δ) with TMS as an internal standard, coupling constants are given in Hz s, singlet, d, doublet; dd, double doublet; m, multiplet.

gynocardin,[8] which has been isolated from some members of the Flacourtiaceae. This glucoside has two hydroxyl groups assigned to carbons 4 and 5 of the cyclopentenyl ring. It will be of interest to establish the configuration at C-1 of these three compounds to reconcile the obvious similarity of the series.

EXPERIMENTAL

Isolation of the Glucosides

Immature fruit of *Tetrapathaea tetrandra* were harvested and frozen with liquid N_2 In a typical extraction the frozen fruit (200 g) were ground to a powder and suspended in $CHCl_3$–MeOH–HCOOH (5:12·3, by vol, 2 l)[9] at −80°. The mixture was warmed to −5°, allowed to stand for 24 hr and filtered, after which the filtrate was separated into two phases[9] by the addition of $CHCl_3$ and water The bottom phase was washed further with water ($\frac{1}{10}$ volume) and the combined aqueous phases were evaporated to dryness under reduced pressure. The residue, dissolved in water (30 ml), was extracted (×8) with *n*-butanol and the butanol extracts were combined and evaporated to dryness under reduced pressure.

To remove phenolic material the extract was washed through Polyclar AT (150 g) with water (750 ml) and the aqueous solution was evaporated to dryness. The residue was absorbed onto silicic acid (10 g) and the dry powder applied to a silicic acid column (150 g, 15% water, Woelm) The column was developed with $CHCl_3$ (100 ml), $CHCl_3$–MeOH, 9 1, v/v, (1 3 l) and $CHCl_3$–MeOH, 4 1, v/v, (2 l) collected as 150 ml fractions. Tetraphyllin A, eluted with $CHCl_3$–MeOH, 9 1, was rechromatographed on Florsil (40 g, Floridin Co) in $CHCl_3$–MeOH, 9 1. This solvent eluted pure tetraphyllin A which was crystallized from EtOAc as needles (0·12 g), m p. 116–118° (Found· C, 53·4; H, 6 4, O, 35 5 $C_{12}H_{17}NO_6$. Required: C, 53 2, H, 6 3; O, 35·4), $[\alpha]_D^{25}$ − 14 0° (C, 1·0 in water)) Tetraphyllin B was eluted with $CHCl_3$–MeOH, 4 1, and crystallized from methanol–EtOAc as needles (0 81 g), m p. 169–170° (Found: C, 50·1; H, 6 0; O, 38·8. $C_{12}H_{17}NO_7$. Required C, 50·1; H, 5 9; O, 39 0), $[\alpha]_D^{25}$ − 35 6° (C, 1 0 in water.)

Acetylation of the Glucosides

Solutions of tetraphyllin A (20 mg) and B (20 mg) in pyridine (4 ml) and Ac_2O (2 ml) were kept at room temp for 24 hr. The mixture was evaporated to dryness under reduced pressure and re-evaporated after addition of ethanol to remove traces of pyridine. The acetates were crystallized from EtOAc–light petroleum Tetraphyllin A-tetraacetate had m p. 108–109°, $[\alpha]_D^{25}$ − 2 0° (C, 1 0 in $CHCl_3$) while tetraphyllin B-pentaacetate had m p 114–115°, $[\alpha]_D^{25}$ − 25° (C, 1 0 in $CHCl_3$).

Tetraphyllin A-tetraacetate (50 mg) in CCl_4 (10 ml) was refluxed with *N*-bromosuccinimide (50 mg) for 1 hr. The solution was cooled, the succinimide filtered off and the filtrate evaporated to dryness under reduced pressure The bromo compound was dissolved in HOAc (20 ml) and refluxed with silver acetate (75 mg). The solution was cooled, filtered and the filtrate evaporated to dryness under reduced pressure to give a colourless glass (55 mg). It gave one peak on GLC (6 ft × 4 mm column packed with 1% SE 30 on Gas Chrom Q, 200°, N_2 60 ml/min) and one spot on TLC ($CHCl_3$–benzene, 9·1) with the same retention time (9 25 min) and R_f (0 11) respectively, as tetraphyllin B-pentaacetate.

Hydrolysis of the Glucosides

(a) *Enzymic* Cyanogenic compounds in fresh tissue were determined by either autolysing the tissue with $CHCl_3$, or by grinding the tissue in liquid N_2 and then adding toluene. The cyanide released was trapped in 1N NaOH (0 5 ml) and estimated by the method of Aldridge [10] The cyanogenic compounds in the extracts and the fractions eluted from columns were detected by releasing HCN (picric acid paper) with linamarase in pH 6 2 phosphate buffer. Where appropriate HCN was identified with the ferricyanide test [11] Tetraphyllin A and B were hydrolysed for 2 days at 30° in a centre-well flask containing phosphate buffer (1·5 ml, 0·02 M, pH 7 0) isopropanol (0 4 ml) and linamarase solution (0 1 ml, ethanol fractionated).[12] The centre well contained NaOH (0 5 ml, 1N) which trapped the released cyanide and this was determined as above.[10] The hydrolysate was deionized with a mixed bed resin and the glucose identified by PC (1 solvent) and by oxidation with glucose oxidase.

(b) *Acidic.* Tetraphyllin A (100 mg) was hydrolysed with 1N H_2SO_4 (2 ml) for 2 hr at 100° in a sealed tube. Extraction of the hydrolysate with ether gave, after evaporation to dryness, an oil (33 mg). GLC (6 ft × 4 mm column packed with 12% EGS on Gas Chrom Q, 100°, N_2 60 ml/min) of this oil showed two peaks with the major component of longer retention time (34 4 min) This adduct had ν_{max} ($CHCl_3$) 3050,

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[9] R L BIELESKI and R. E. YOUNG, *Anal Biochem* **6**, 54 (1963).
[10] W N ALDRIDGE, *Analyst* **69**, 262 (1944).
[11] A. O GETTLER and L GOLDBAUM, *Anal Chem* **19**, 270 (1947)
[12] I E COOP, *N Z J Sci Technol.* **22**, 71B (1940)

2290, 1660, 860 cm^{-1} in the i.r and showed signals at δ 6 49 (2H, m), 4 91 (2H, m), 2 54 (2H, m), 2·0 (4H, m) in the NMR spectrum ($CDCl_3$).

Tetraphyllin B (100 mg) was hydrolysed with 1N H_2SO_4 (2 ml) for 2 hr at 100° in a sealed tube. The hydrolysate was neutralized with $BaCO_3$, filtered and the filtrate evaporated to dryness under reduced pressure. Chromatography on silicic acid (10 g) with $CHCl_3$–MeOH, 20·1 (v/v) gave 4-hydroxy-2-cyclopenten-1-one as an oil (19 mg, 68% yield), $[\alpha]_D^{25}$ − 28 2° (C, 1 in water), λ_{max} (EtOH) 213 nm, ϵ 6200; ν_{max} ($CHCl_3$) 3350, 1720 cm^{-1}; 2,4-dinitrophenylhydrazone m.p. 109–110°.

Glucose was identified in the hydrolysates of tetraphyllin A and B by PC in three solvents.

(c) *Alkaline* Tetraphyllin B (500 mg) was hydrolysed on a steam bath for 1 hr with $Ba(OH)_2$ (1 2 g) in water (8 5 ml). CO_2 was bubbled into the solution and the precipitate removed by centrifugation. The supernatant was treated with Dowex 50 (H^+) resin (3·6 g) and evaporated to dryness to give tetraphyllinic acid B as a clear oil, ν_{max} (liq. film) 3300, 1730 cm^{-1}. The acid was treated with CH_2N_2 in ether and chromatographed on silicic acid (30 g, 15% water) in $CHCl_3$–MeOH, 9·1 (v/v) This solvent eluted the methyl ester of tetraphyllinic acid B, as an oil (0 3 g, 57% yield), $[\alpha]_D^{25}$ − 48 6° (C, 1 in water), ν_{max} (liq. film) 3300, 1730 cm^{-1}.

The methyl ester was hydrolysed in 0 02 M phosphate buffer (pH 6 2, 100 ml) with linamarase solution (5 ml) at 30° for 24 hr, after which the hydrolysate was treated with mixed bed resin [Dowex 50 (H^+), Dowex 1 (HCO_3^-)] and evaporated to dryness under reduced pressure. Chromatography on silicic acid (40 g, 15% water) in $CHCl_3$–MeOH, 9.1 (v/v) gave methyl-1,4-dihydroxy-2-cyclopenten-1-carboxylate as an oil (58 mg, 48% yield), $[\alpha]_D^{25}$ + 9 3° (C, 0 5 in water), ν_{max} ($CHCl_3$) 3650, 1750 cm^{-1}.

Tetraphyllin A (100 mg) was hydrolysed with $Ba(OH)_2$ as above. The carboxylic acid obtained was hydrolysed with 0 5 N H_2SO_4 (2 ml) for 1 hr in a sealed tube to give a solid precipitate which was recovered and recrystallized from acetone–MeOH–water. The crystalline adduct (15 mg) had m.p 220° (with sublimation), ν_{max} ($CHCl_3$) 1700, 1300 cm^{-1} and gave a methyl ester on treatment with CH_2N_2, m.p. 172–174°, ν_{max} ($CHCl_3$) 1730, 1250 cm^{-1}. The NMR spectrum ($CDCl_3$) of the methyl ester showed signals at δ 6 40 (2H, m), 4 13 (1H, dd, $J = 9$ Hz $J' = 4$ Hz), 4 65 (1H, dd, $J = 9$ Hz $J' = 1$ Hz), 3 60 (6H, s), 3 05 (2H, m), 2 22 (4H, m)

Chromatography

The glucosides were chromatographed on thin layers of silica gel with $CHCl_3$–ethanol (95%) (4.3, v/v) and were detected with H_2SO_4–H_2O (1:1, v/v) spray. They were also chromatographed on cellulose thin layers with acetone–2-butanone–water (5:12:3, by vol) and detected by the method of Bennett and Tapper.[13]

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[13] W. D. Bennett and B. A. Tapper, *J. Chromatog.* **34**, 428 (1968).