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SHORT REPORTS

SULPHATE ESTERS OF CYCLOPENTENOID CYANOHYDRIN GLYCOSIDES*

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Key Word Index—Passifloraceae; sulphate esters; cyanohydrin glucosides; tetraphyllin B.

Abstract—A sulphate ester of tetraphyllin B, (1*S*, 4*S*)-1-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclopentene-1-carbonitrile-4-*O*-sulphate, was isolated from four *Passiflora* species (*P. caerulea*, *quadrangularis*, *racemosa* and *hybrida*) by anion exchange. The structural assignment is founded on sulphation-induced shifts in ^{1}H and ^{13}C NMR spectra, negative-ion FABMS, and acid-catalysed desulphation to tetraphyllin B.

INTRODUCTION

Sulphate esters are well known from plants as glucosinolates, sulphated flavonoids, and algal polysaccharides [2–5]. However, the occurrence of active sulphate, 3′-phosphoadenosine-5′-phosphosulphate is ubiquitous in plants [6, 7], and synthesis of sulphate esters within other major groups of natural products is perhaps more frequent than currently documented [8–12]. In 1979, Hübel and Nahrstedt isolated the first sulphated cyanohydrin glycoside [13]. More recently, Seigler *et al.* discovered sulphated cyanohydrin glycosides with a cyclopentene ring [14, 15]. The latter class is the subject of the present work.

RESULTS AND DISCUSSION

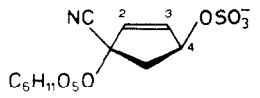
Passiflora quadrangularis L., *P. racemosa* Brot. and *P. hybrida* hort., cultivar Imp. Eugénie, have long been known to produce cyanide in significant amounts [16–20]. Preliminary investigation of the extracts (TLC) demonstrated the presence of a very polar cyanogenic constituent in each case; since sulphate esters were anticipated, it appeared most logical to employ anion exchange for purification [21, 22]. Thus, the cyanogenic material was quantitatively retained on aminopropylsilica (Nucleosil NH_2) and eluted from the column with ammonium acetate, after which the product was desalted by repeated freeze-drying and converted to a potassium salt by cation exchange.

Investigation of the materials so obtained by ^{1}H NMR (500 MHz, CD_3OD) revealed the presence of the same cyclopentenoid cyanohydrin glucoside in each case, with the characteristic spin patterns of a 1,1,4-trisubstituted 2-cyclopentene and of a β-D-glucopyranosyloxy unit, similar to those described before [23, 24]. Significantly, the characteristic multiplet of the allylic proton (H-4) appeared at δ5.52, whereas in cyclopentenoids bearing a free hydroxy group at C-4 this resonance appears at δ4.98 (*trans*-1,4-dioxygenation of the ring [23]) or at δ4.81 (*cis*-1,4-dioxygenation [24]). The downfield shift of H-4 was paralleled by a downfield shift of C-4 (δ81.9). The expected sulphate group was hence located at this site.

The glucoside was inert towards purified sulphatase from limpets (*Patella vulgata*), whereas a sulphatase preparation from *Helix pomatia*, well-known for its β-glucosidase activity [25, 26], cleaved the glucosidic bond at least as effectively as the sulphate group (TLC). Consequently, the sulphate group was removed by brief heating of the acid form of the compound [27]. The glucoside formed upon desulphation was tetraphyllin B [23], as shown by 500 MHz ^{1}H NMR data (free glucoside and its pentaacetate). Sulphate formed in the hydrolysis was identified by precipitation with barium ion. The parent glucoside thus has the structure **1**, in agreement with M_r determined by negative-ion FABMS (*m/z* 366, $[M]^-$). The laevorotation of the glucoside ($[M]_D$ −145°) neatly confirms the (*S*) configuration of C-4 [23].

In contrast to previously studied glucosides (deidaclin and tetraphyllin A [28], volkenin and tetraphyllin B [29], taraktophyllin and epivolkenin [24]), which appear always to occur as pairs of glucosides with enantiomeric aglucones [1], the sulphate ester **1** was in the present work found as a single isomer. In a report on a related species, *Passiflora caerulea* L., Seigler *et al.* claimed identification of two sulphate esters epimeric at C-1 [14]. The alleged mixture of epimeric sulphate esters was reported to give in D_2O a ^{1}H NMR spectrum in which all resonances of the components exactly coincide [14]; only by redetermination of the spectrum in a CD_3OD–D_2O mixture was the presence of two isomers apparent [14]. However, from the reported chemical shifts in D_2O and from the spectrum of the acetylated product [14] it is clear that the sample studied was tetraphyllin B. The sample used for determination of the spectrum in CD_3OD–D_2O was different and appears in fact to have contained **1**, as concluded from comparison of the re-

*Part 10 in the series 'Cyclopentenoid Cyanohydrin Glycosides'. For part 9 see ref. [1]



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ported ^{1}H NMR data [14] with the data obtained in the present work. The identity of the second compound of Seigler *et al.* [14] is not derivable from the data reported. We have reinvestigated the cyanogenesis of *P. caerulea* and found that it indeed contains **1**, in our case unaccompanied by any isomer, analogue, or artifact. The identity of another sulphated cyclopentenoid cyanohydrin glycoside detected by Spencer and Seigler in *P. coccinea* [15] has yet to be ascertained.

EXPERIMENTAL

General methods were as previously described [1, 23]. FAB mass spectra were recorded on a Kratos MS50-RF operating in the negative mode. The plant material was obtained from the Botanical Garden, University of Copenhagen, Copenhagen. Fresh leaves of *P. caerulea* L. (96 g), *P. quadrangularis* L. (73 g), *P. racemosa* Brot. (213 g) and *P. hybrida* Imperatrice Eugénie (34 g) were extracted and the extracts initially fractionated on silica gel in the usual manner [1].

The cyanogenic fractions were evapd, dissolved in H_2O, centrifuged and injected onto a 0.8 × 25 cm HPLC column of Nucleosil NH_2 (5 μm), previously equilibrated with 0.5 M aq. $MeCOONH_4$ and rinsed with H_2O. The column was washed with H_2O (50 ml, 2 ml/min; the eluate evapd and the residue found to contain no cyanogenic constituents), and the cyanogenic material desorbed with 60 ml 0.5 M aq. $MeCOONH_4$ (2 ml/min). The eluate was repeatedly freeze-dried, each time adding a fresh portion of H_2O. The colourless powder was dissolved in H_2O and passed through a bed (10 ml) of Dowex 50W (K^+ form) and freeze-dried. The yields (glassy syrup) were: *P. caeruela* 160 mg (0.17% of fresh weight), *P. quadrangularis* 94 mg (0.13%), *P. racemosa* 272 mg (0.13%), *P. hybrida* 65 mg (0.19%). All four samples gave identical ^{1}H NMR spectra (500 MHz, CD_3OD): δ2.59 and 2.97 ($^2J_{AB}$ −15.1 Hz, $^3J_{AX}$ 3.5 Hz, $^3J_{BX}$ 6.8 Hz; H-5A and H-5B), 3.22 ($^3J_{1,2}$ 7.8 Hz, $^3J_{2,3}$ 9.1 Hz; H-2′), 3.30–3.45 (unresolved; H-3′, H-4′, H-5′), 3.72 and 3.87 ($^2J_{AB}$ −12.2 Hz, $^3J_{AX}$ 4.8 Hz, $^3J_{BX}$ 1.8 Hz; H-6′A and H-6′B), 4.57 ($^3J_{1,2}$ 7.8 Hz; H-1′), 5.52 (H-4), 6.29 and 6.46 ($^3J_{AB}$ 5.6 Hz, J_{AX} 1.2 Hz, J_{BX} 2.2 Hz; H-2 and H-3). Measurements and reactions reported below were carried out with the material from *P. racemosa*.

^{1}H NMR (250 MHz, D_2O): δ2.72 and 2.92 ($^2J_{AB}$ −15.3 Hz, $^3J_{AX}$ 3.3 Hz, $^3J_{BX}$ 6.5 Hz; H-5A and H-5B), 3.26 (H-2′), 3.35–3.53 (H-3′, H-4′, H-5′), 3.72 and 3.89 ($^2J_{AB}$ −12.5 Hz, $^3J_{AX}$ 5.0 Hz, $^3J_{BX}$ 2.0 Hz; H-6′A and H-6′B), 4.71 (H-1′, $^3J_{1,2}$ 7.8 Hz), 5.55 (H-4), 6.36 and 6.50 ($^3J_{AB}$ 5.6 Hz, J_{AX} 1.0 Hz, J_{BX} 2.2 Hz; H-2 and H-3); ^{13}C NMR (62.9 MHz, CD_3OD): δ46.3 (C-5), 62.5 (C-6′), 71.2 (C-4′), 74.7 (C-2′), 77.9 and 78.2 (C-3′ and C-5′), 81.9 (C-4), 82.2 (C-1), 120.1 (CN), 134.3 and 140.9 (C-2 and C-3); IR (KBr): ν_{max} 3400 (strong, *br*; OH), 1625 (weak; C=C), 1240 (strong; S=O); $[\alpha]_D^{25}$ −36° (c 0.1, H_2O), $[M]_D^{25}$ −145°. FAB MS (Xe, 9.5 keV, glycerol): *m/z* 366 $[M]^-$.

An aq. soln of the potassium salt (10 mg) was passed through Dowex 50 W (H^+ form), boiled for 15 min, chilled on ice, and freeze-dried, and the residue chromatographed on LiChrosorb RP-18 in the usual way [1, 23], to give a band with R_f corresponding to tetraphyllin B. Selected ^{1}H NMR signals (250 MHz, CD_3OD): δ2.22 and 2.91 (H-5), 4.48 (H-1′), 4.97 (H-4), 6.15 and 6.31 (H-2 and H-3) [23]. The material was acetylated in the usual manner; selected ^{1}H NMR signals (250 MHz, $CDCl_3$): δ2.46 and 2.86 (H-5), 4.88 (H-1′), 5.74 (H-4), 6.07 and 6.33 (H-2 and H-3) [23].

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