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Natural Glycosides Containing Allopyranose from the Passion Fruit Plant and Circular Dichroism of Benzaldehyde Cyanohydrin Glycosides[1]

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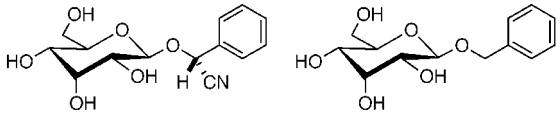
ABSTRACT



1 **2**

Leaves of the edible passion fruit plant, *Passiflora edulis*, contain benzylic β-D-allopyranosides 1 and 2, representatives of a rare class of natural glycosides with D-allose as the only sugar constituent. The glycoside 1 is the first known cyanogenic glycoside containing a sugar different from D-glucose attached directly to the cyanohydrin center. Asymmetric perturbation of the ^1L_b transition of the benzene chromophore was shown to be useful for determination of absolute configuration of the cyanohydrin center of aromatic cyanogenic glycosides.

Glycosides form a very large and diverse group of natural products, contributing to the immense chemical diversity of secondary plant metabolites.[2] A vast majority of natural glycosides contain β-D-glucopyranosyl as the only sugar residue and are formed by a direct glucosyl transfer[3,4] from uridinediphosphoglucose (UDP-glucose) to a variety of aglycones, in particular with phenylpropanoid, polyacetate, or terpenoid structure.[2] Sometimes, a second sugar different from D-glucose is attached to one of the hydroxy groups of the glucose residue. However, glycosides containing a single monosaccharide residue other than β-D-glucopyranose are very rare.[2] This demonstrates the universal occurrence of the glucosylation mechanism employing UDP-glucose in plants.

Herein, we report on the isolation of two new natural products bearing the β-D-allopyranose residue, **1** and **2**, along with the classical[5] β-D-glucopyranoside, prunasin (**3**), from leaves of *Passiflora edulis* Sims (Passifloraceae), the plant that yields the edible passion fruit. The glycosides **1** and **3** are cyanogenic glycosides,[5] representing a group of natural products which is of pertinent interest as antinutritional plant constituents[6] and as chemicals involved in plant−insect interactions.[7] The ratio between **1** and **3** in the plant material was 4:1. The presence of cyanogenic glycosides derived from phenylalanine in *Passiflora edulis*[8] is unexpected, as aliphatic glycosides derived from valine, isoleucine, and 2-cyclopentenylglycine are characteristic cyanogens of the plant family Passifloraceae.[9,10] Moreover, **1** is the first cyanogenic β-D-

(1) Part 22 in the series. For part 21, see: Wellendorph, P.; Clausen, V.; Jørgensen, L. B.; Jaroszewski, J. W. *Biochem. Syst. Ecol.* **2001**, *29*, 649−651.

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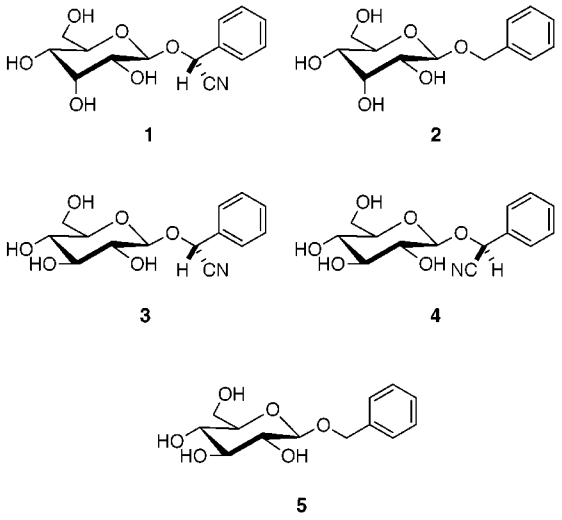
allopyranoside to be described[11] and indeed the only known cyanogenic glycoside containing a single sugar residue different from D-glucose.[5] In accordance with tradition followed within the field, we propose the name passiedulin for the new glycoside **1**.



The structures **1** and **2** follow readily from NMR spectroscopic data.[12,13] Thus, the ^{1}H NMR spectra show spin−spin coupling patterns of a β-hexopyranose moiety with H-1, H-2, H-4, and H-5 axial and H-3 equatorial. That the configuration of the cyanohydrin center of **1** is the same as that of **3**, i.e., (*R*),[5] was evident from the similarity of their CD spectra, which showed positive Cotton effects at 255−270 nm (Figure 1). By contrast, the CD spectrum of

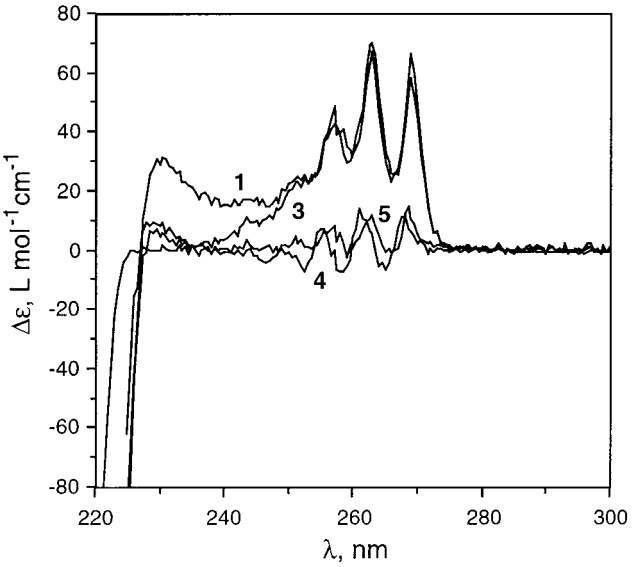


Figure 1. CD spectra (methanol, ambient temperature) of passiedulin (**1**), prunasin (**3**), sambunigrin (**4**), and benzyl D-glucopyranoside (**5**).

sambunigrin (**4**), which has the (*S*) configuration of the cyanohydrin center,[5] was different, being akin to that of

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(11) Other known examples of natural β-D-allopyranosides include an iridoid glycoside together with its several acetylated derivatives,[11a−c] alloside of phenylethanol and its derivatives bearing caffeoyl residue in the sugar part,[11d−f] alloside of hydroquinone and its caffeoyl derivatives,[11g,h] one chromone,[11i] three cardenolides,[11j,k] 4-(β-D-allopyranosyloxy)phenylmethanol and its derivatives,[11l,m] two terpenoids,[11n,o] and a number of flavonoids.[11p−x] (a) Jensen, S. R.; Nielsen, B. J.; Norn, V. *Phytochemistry* **1985**, *24*, 487−489. (b) Hase, T.; Iwagawa, T.; Dave, M. N. *Phytochemistry* **1985**, *24*, 1323−1327. (c) Bock, K.; Jensen, S. R.; Nielsen, B. J.; Norn, V. *Phytochemistry* **1978**, *17*, 753−757. (d) Toyota, M.; Saito, T.; Asakawa, Y. *Phytochemistry* **1996**, *43*, 1087−1088. (e) Jensen, S. R. *Phytochemistry* **1996**, *43*, 777−783. (f) Wada, H.; Shimizu, Y.; Tanaka, N.; Cambie, R. C.; Braggins, J. E. *Chem. Pharm. Bull.* **1995**, *43*, 461−465. (g) Machida, K.; Nakano, Y.; Kikuchi, M. *Phytochemistry* **1991**, *30*, 2013−2014. (h) Iwagawa, T.; Takahashi, H.; Munesada, K.; Hase, T. *Phytochemistry* **1984**, *23*, 468−469. (i) Tanaka, N.; Maehashi, H.; Saito, S.; Murakami, T.; Saki, Y.; Chen, C.-M.; Iitaka, Y. *Chem. Pharm. Bull.* **1979**, *27*, 2874−2876. (j) Ferth, R.; Kopp, B. *Pharmazie* **1992**, *47*, 626−629. (k) Kopp, B.; Kubelka, W. *Planta Medica* **1982**, *45*, 87−94. (l) Shide, L.; Rücker, G. *Planta Med.* **1986**, 412. (m) Perold, G. W.; Beylis, P.; Howard, A. S. *J. Chem. Soc., Perkin Trans. 1* **1973**, 643−648. (n) Redgwell, R. J.; Beever, R. E.; Bielski, R. L.; Laracy, E. P.; Benn, M. H. *Carbohydr. Res.* **1990**, *198*, 39−48. (o) Zdero, C.; Bohlmann, F. *Phytochemistry* **1989**, *28*, 2745−2751. (p) Khamidullina, E. A.; Gromova, A. S.; Lutsky, V. I.; Semenov, A. A.; Li, D.; Owen, N. L. *Russ. Chem. Bull.* **1999**, *48*, 390−392. (q) Das, K. C.; Tripathi, A. K. *Fitoterapia* **1997**, *68*, 477. (r) Liu, S.; Xiao, Z.; Feng, R. *Phytochemistry* **1994**, *35*, 1595−1596. (s) Hasan, A.; Farman, M.; Ahmed, I. *Phytochemistry* **1994**, *35*, 275−276. (t) Iwashina, T.; Ootani, S. *Phytochemistry* **1990**, *29*, 3639−3641. (u) Cui, C.-B.; Tezuka, Y.; Kikuchi, T.; Nakano, H.; Park, J.-H. *Chem. Pharm. Bull.* **1990**, *38*, 2620−2622. (v) Hwang, T.-H.; Kashowada, Y.; Nonaka, G.-I.; Nishioka, I. *Phytochemistry* **1989**, *28*, 891−896. (w) Okuyama, T.; Hosoyama, K.; Hiraga, Y.; Kurono, G.; Takemoto, T. *Chem. Pharm. Bull.* **1978**, *26*, 3071−3074. (x) Shizmu, E.; Tomimatsu, T.; Nohara, T. *Chem. Pharm. Bull.* **1984**, *32*, 5023−5026.

(12) Passiedulin (**1**) (23 mg isolated from 147 g of dry plant material by silica gel chromatography and repeated preparative HPLC on a C_{18} phase with H_2O/CH_3OH 7:3): HR MALDI FT MS *m/z* (rel intensity) 318.0958 (100%, $[MNa]^+$), $[C_{14}H_{17}NO_6 + Na]^+$ requires 318.0948; $[\alpha]^{25}_D$ −69° (*c* 0.24, methanol); ^{1}H NMR (400 MHz, CD_3OD) δ 3.42 (1H, dd, J = 7.9 and 3.0 Hz, H-2′), 3.49 (1H, dd, J = 9.5 and 2.9 Hz, H-4′), 3.63−3.71 (2H, m, H-5′ and H-6′A), 3.85−3.92 (1H, m, H-6′B), 4.02 (1H, t, J = 3.0 Hz, H-3′), 4.61 (1H, d, J = 7.9 Hz, H-1′), 5.90 (1H, s, benzylic H), 7.43−7.47 (3H, m) and 7.50−7.56 (2H, m) (aromatic protons) (the assignment is based on COSY correlations); ^{13}C NMR (100 MHz, CD_3OD) δ 63.2 (C-6′), 68.5 (benzylic C), 68.9 (C-4′), 72.1 (C-2′), 73.0 (C-3′), 75.9 (C-5′), 100.1 (C1′), 119.7 (CN), 129.0 and 130.2 (*ortho* and *meta* C), 130.9 (*para* C), 135.2 (*ipso* C) (the assignment is based on a ^{1}H,^{13}C-correlation). Passiedulin tetraacetate (obtained by overnight treatment of 2 mg of **1** with acetic anhydride and pyridine, 1:1): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00, 2.01, 2.11 and 2.12 (acetyl CH_3), 4.05 (1H, o, J = 10.0, 4.5 and 2.7 Hz, H-5′), 4.18−4.26 (2H, m, H-6′), 4.89 (1H, d, J = 8.2 Hz, H-1′), 4.96−5.00 (2H, m, H-2′ and H-4′), 5.53 (1H, s, benzylic H), 5.67 (1H, t, J = 3.0 Hz, H-3′), 7.43−7.50 (5H, m, aromatic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5 (2C), 20.7 and 20.8 (acetyl CH_3), 62.1 (C-6′), 66.0 (C-4′), 68.2 (C-3′), 68.7 (benzylic C), 68.8 (C-2′), 70.6 (C-5′), 97.3 (C-1′), 116.9 (CN), 127.5 and 129.1 (*ortho* and *meta* C), 130.2 (*para* C), 132.5 (*ipso* C), 168.8, 169.0, 169.6 and 170.8 (acetyl CO). Voucher specimen (DFHJJ9) of the plant used in this work was deposited in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen).

(13) Compound **2** (4 mg): HR MALDI FT MS *m/z* (rel intensity) 293.0988 (100%, $[MNa]^+$), $[C_{13}H_{18}O_6 + Na]^+$ requires 293.0996; ^{1}H NMR (400 MHz, CD_3OD) δ 3.37 (1H, dd, J = 7.9 and 3.0 Hz, H-2′), 3.49 (1H, dd, J = 9.5 and 3.0 Hz, H-4′), 3.64−3.72 (2H, m, H-5′ and H-6′A), 3.85−3.92 (1H, m, H-6′B), 4.02 (1H, t, J = 3.0 Hz, H-3′), 4.72 (1H, d, J = 7.9 Hz, H-1′), 4.64 and 4.92 (each 1H, d, J = 12.0 Hz, benzylic CH_2), 7.27 (1H, distorted t, H-4), 7.32 (2H, distorted t, H-3 and H-5), 7.41 (2H, distorted d, H-2 and H-5); ^{13}C NMR (100 MHz, CD_3OD) δ 63.2 (C-6′), 69.1 (C-4′), 71.8 (benzylic C), 72.5 (C-2′), 73.0 (C-3′), 75.6 (C-5′), 101.0 (C-1′), 128.7−129.3 (aromatic).

benzyl β-D-glucopyranoside (**5**) (Figure 1).[14] The CD spectrum of **2** (not shown) was closely similar to that of **5**.

Cotton effects at about 255−270 nm in aromatic compounds[15] are associated with transition from the lowest energy vibrational mode in the electronic ground state to the fully symmetric vibrational modes in the ^1L_b electronically excited state of the benzene chromophore and are the result of vibrational borrowing from allowed ^1L_a transitions.[16] The observed Cotton effects in the CD spectra of **1**, **3**, and **4** (Figure 1) appear to originate from chiral perturbations caused not only by the cyanohydrin center, which would be expected to be the dominating contribution, but also by the sugar residue. The existence of the latter interaction is apparent in the spectrum of **5** (Figure 1). Although chiral perturbation of the benzene chromophore by chiral centers separated from the benzene ring by a methylene group has been extensively studied and shown to be useful for configurational assignments,[17] the CD spectrum of benzyl β-D-glucopyranoside (**5**), in which the chiral center is three bonds away from the benzene chromophore, has to our knowledge not been reported prior to this work. The similarity of the CD spectra of **4** and **5** is surprising and appears to reflect a diminished contribution of the (*S*)-cyanohydrin center in **4**, presumably owing to conformational effects. On the other hand, the similarity of the spectra of **1** and **3** demonstrates the lack of significant influence of the orientation of the C-3′ hydroxy group in the (*R*)-series. Thus, we expect that Cotton effects associated with the ^1L_b transition in benzaldehyde cyanohydrin glycosides may be useful for configurational assignments irrespective of the nature of the sugar residue present, as long as the absolute configuration of the anomeric carbon and conformation of the glycosidic bond remain the same.[18]

The reported presence of passiedulin (**1**) and **2** in *Passiflora edulis* raises several questions in the context of biochemistry and biology of cyanogenic glycosides. The function of a β-D-glucopyranoside such as **3** as a plant defense substance depends on the presence in the same plant tissue of an often highly specialized β-glucosidase.[5,19] The enzyme catalyses hydrolysis of the cyanogenic glycoside with formation of hydrogen cyanide. Whether passiedulin (**1**) can be regarded as a defense compound depends on whether *Passiflora edulis* contains an allosidase capable of releasing cyanide from **1**. In principle, **1** and **2** may be formed by enzymatic epimerization of the corresponding β-D-glucopyranosides, or via D-allopyranosyl transfer to the corresponding alcohols from UDP-allose. These points and the possible evolutionary advantage of accumulating **1** as opposed to **3** have yet to be clarified.

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(14) Sambunigrin (**4**) was isolated from *Sambucus nigra* L. (Caprifoliaceae),[14a] and benzyl β-D-glucopyranoside (**5**) was synthesized by enzymatic, nonaqueous glucosylation of benzyl alcohol catalyzed by almond emulsin.[14b,c] (a) Jensen, S. R.; Nielsen, B. J. *Acta Chem. Scand.* **1973**, *27*, 2661−2662. (b) Vic, G.; Crout, D. H. G. *Carbohydr. Res.* **1995**, *279*, 315−319. (c) Vic, G.; Thomas, D. *Tetrahedron Lett.* **1992**, *33*, 4567−4570.

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(18) At present, configurational assignment of the cyanohydrin center in benzaldehyde cyanohydrin glycosides is often still carried out using classical, empirical correlations of chromatographic elution orders[18a,b] or NMR chemical shifts.[18c,d] Although useful,[8a] these methods require access to both epimers of the glycoside and are of unproven utility in the case of the presence of novel sugars, as in **1**. (a) Nahrstedt, A. *J. Chromatogr.* **1970**, *50*, 518−520. (b) Nahrstedt, A. *J. Chromatogr.* **1978**, *152*, 265−268. (c) Schwarzmaier, U. *Chem. Ber.* **1976**, *109*, 3250−3251. (d) Hübel, W.; Nahrstedt, A.; Wray, V. *Arch. Pharm. (Weinheim)* **1981**, *314*, 609−617.

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