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THE ABSOLUTE CONFIGURATIONS OF THE PHENOLIC CYANOGENETIC GLUCOSIDES TAXIPHYLLIN AND DHURRIN

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Abstract—A glucoside of *p*-hydroxymandelonitrile has been isolated from *Taxus canadensis*, *T. media*. var *hicksii* and *T. cuspidata* var. *nana* and shown to be identical with phyllanthin. Since the name phyllanthin has been used for more than one compound the alternative name taxiphyllin is proposed. Proton magnetic resonance (PMR) spectral studies of fully acetylated taxiphyllin, dhurrin and prunasin indicate that taxiphyllin is β-D- glucopyranosyloxy-D-*p*-hydroxymandelonitrile and that dhurrin is β-D-glucopyranosyloxy-L-*p*-hydroxymandelonitrile. These conclusions are supported by conformational analysis and specific rotation data.

ALTHOUGH cyanogenetic glycosides have been isolated from angiosperms[1] their occurrence has not previously been reported in gymnosperms. It is known, however, that yew leaves are cyanophoric since emulsin hydrolysis of an aqueous extract can yield as much as 0·1% of the fresh weight of the leaves as hydrogen cyanide.[2] The purpose of this communication is to report the isolation of a β-D-glucoside of *p*-hydroxymandelonitrile, from the leaves of *Taxus* × *T. media* Rehder var. *hicksii* Rehder, *T. canadensis* Marsh and *T. cuspidata* S. & Z. var. *nana* Rehder, and to offer evidence in support of the absolute configuration at the asymmetric centre of the aglycon. The compound has been given the trivial name taxiphyllin.

Three β-D-glucosides in the mandelonitrile series have been isolated,[1] namely the two possible diastereoisomers and a mixture of the latter. These compounds were named prunasin, sambunigrin and prulaurasin, respectively. It has been shown that acid hydrolysis of sambunigrin yields (+) mandelic acid[3] which has the L-configuration at the asymmetric centre of the aglycon.[4] Consequently, since prunasin yields (−) mandelic acid on acid hydrolysis[3,5] the aglycon of this compound must have the D-configuration. Prulaurasin was shown to be a mixture of prunasin and sambunigrin.[6] Considerable confusion exists in the literature, on the other hand, concerning the naturally occurring β-D-glucosides of the *p*-hydroxymandelonitrile series. The first compound of this type was isolated from *Sorghum vulgare* and was given the name dhurrin.[7] This compound was incompletely characterized and the configuration of the asymmetric centre of the *p*-hydroxymandelonitrile moiety was not established.

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[3] E. Bourquelot and H. Herissey, *Compt. rend. Soc. Biol.* **62**, 828–829 (1907).
[4] K. Mislow, *J. Amer. Chem. Soc.* **73**, 3954–6 (1951).
[5] E. Fischer, *Ber. Dtsch. Chem. Ges.* **28**, 1508 (1895).
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Indeed the only pertinent data reported for the compound were an elemental analysis, an indefinite melting point of 100–200°, and that emulsin hydrolysis gave *p*-hydroxybenzaldehyde, hydrogen cyanide and glucose. Consequently, when a similar glucoside was isolated in Australia from *Phyllanthus gastroemii* the workers were unable to establish whether this compound was identical to dhurrin, and named it phyllanthin.[8] The selection of this name was unfortunate since it has been used earlier for other compounds isolated from *Phyllanthus niruri*.[9] Phyllanthin was reported to melt with decomposition at 167° and had an $[\alpha]_D^{20}$ −65°. Furthermore, it was reported to form a tetraacetate which had a melting point of 144°. Their experimental data, however, suggests that they had actually isolated the corresponding pentaacetate derivative.

Although the specific rotations of the two enantiomorphs of *p*-hydroxymandelic acid [10] have been correlated with their absolute configurations, the configuration at the centre of asymmetry in the aglycon of phyllanthin cannot be deduced since no attempt was made to convert it to the corresponding acid.[8] Consequently, it was necessary to establish the relationship of the cyanogenetic glucoside from *Taxus* to dhurrin and phyllanthin, and to determine the configurations of any centres of asymmetry in their aglycons.

Taxiphyllin was found to be $C_{14}H_{17}O_7N$ and to yield hydrogen cyanide, glucose and *p*-hydroxybenzaldehyde as the only products of hydrolysis with emulsin. Therefore, it was assumed to be a β-D-glucoside of *p*-hydroxymandelonitrile in which the cyanohydrin hydroxyl group is involved in the glucosidic linkage. The UV and IR spectra of this compound were consistent with the suggested structure. Acetylation of taxiphyllin with acetic anhydride in pyridine gave in quantitative yield a crystalline pentaacetate with the same melting point as that reported for phyllanthin pentaacetate.[8] Moreover, the specific rotation of taxiphyllin was in good agreement with that of phyllanthin.[8] It seems highly probable, therefore, that the two compounds are identical. In view of the possible confusion from use of the name phyllanthin we suggest that the trivial name for this compound should be taxiphyllin. Final proof of the structure of taxiphyllin was obtained from the PMR spectrum of its pentaacetate derivative in deuterated chloroform (Fig. 1). Four aromatic protons appeared as a characteristic A_2B_2 multiplet at an average τ values of 2·69 which was consistent with the presence of a 1,4-disubstituted benzene ring.[11,12a] The methine hydrogen atom of the aglycon should appear as a singlet at low field since the other three substituents on the same carbon atom (—O—, —C≡N, $-H_4C_6OCOCH_3$) would deshield the proton nucleus,[12b] and do not bear hydrogen atoms capable of spin-spin coupling with it. Consequently, the singlet at 4·46 could be assigned to the proton at the asymmetric centre of the aglycon. The hydrogen atoms on carbons 2, 3 and 4 of the glucose moiety gave a multiplet at an average τ value of 4·91, the proton at carbon 5 at 6·32, and the methylenic protons at carbon 6 at 5·80. An ill-defined doublet with an average spacing of 7 c/s was obtained for the anomeric hydrogen at an average

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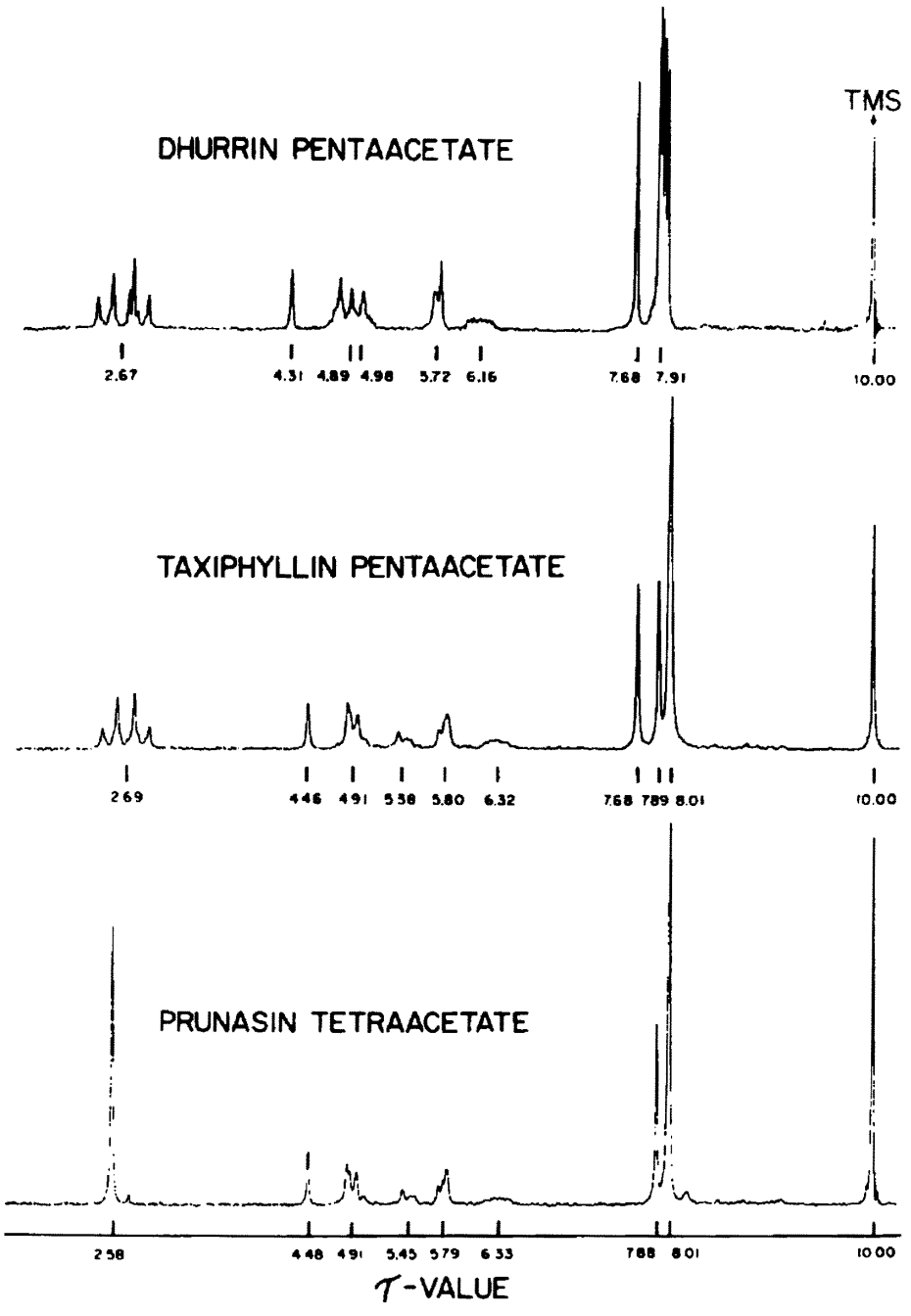

FIG. 1. The PMR spectra of fully acetylated cyanogenetic glucosides in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard.

value of 5·38. The latter values were in good agreement with the average chemical shift of 5·48, and spacing of 7·5 c/s, which was also found for the anomeric hydrogen of methyl, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside. Spacings of 6–9 c/s for the anomeric doublet are characteristic of diaxially orientated hydrogen atoms on carbons 1 and 2 of the glycopyranosides.[13] Consequently, taxiphyllin must be a β-D-glucopyranoside. The presence of three protons of a phenolic acetoxy group[14] at 7·68 provided further evidence that the phenolic hydroxy group is not involved in the glucosidic linkage. Absorption of energy at 7·89 and 8·01 for twelve protons could be assigned to the four acetoxyl groups[15] on the glucose moiety.

Dhurrin was isolated from *Sorghum vulgare* to assist in establishing whether taxiphyllin and dhurrin were identical or were diastereoisomers, or if one of them was a mixture of diastereoisomers. Hydrolysis with emulsin yielded the same products as were obtained from taxiphyllin, and elemental analysis showed it to have the same molecular formula. The UV spectrum was similar to that given by taxiphyllin, and the IR spectra of the two compounds only differed in the intensity of some of the absorption bands. Acetylation of dhurrin with acetic anhydride in pyridine gave a crystalline pentaacetate which had a melting point 12° lower, and a specific rotation 28° more negative, than the corresponding derivative of taxiphyllin. Furthermore, a mixed melting point of the two pentaacetates showed a depression of about 15°. Consequently, the two compounds are not identical and must be diastereoisomers or else one of them is a mixture of diastereoisomers. PMR studies finally established the relationship of taxiphyllin to dhurrin. From Fig. 1 it can be seen that although the PMR spectra of the two pentaacetate derivatives are similar the chemical shifts differ for equivalent protons in the two molecules. This is particularly true for the anomeric and methine hydrogen atoms of dhurrin pentaacetate which now appear as a doublet at average values of 4·98 and a singlet at 4·31 as compared with values of 5·38 and 4·46 for taxiphyllin pentaacetate. The spacing for the anomeric doublet of the dhurrin derivative is still 7 c/s indicating that this compound is a β-D-glucopyranoside. Moreover, all five acetoxyl groups of dhurrin pentaacetate ($\tau = 7{\cdot}68$ and 7·91) are resolved whereas those for the taxiphyllin derivative are only partially resolved. Taxiphyllin and dhurrin therefore, must be diastereoisomers since their PMR spectra are not identical and yet each integrates correctly for a single isomer. A mixture of the two compounds would give a PMR spectrum in which two signals would be observed for the anomeric and methine hydrogen atoms.

Examination of the literature[16] reveals that in the fully acetylated derivatives of the mandelonitrile-β-D-glucosides the D-isomer, prunasin tetraacetate (m.p. 139–140°, $[\alpha]_D$ −24°), has a higher melting point and a lower specific rotation than the L-isomer, sambunigrin tetraacetate (m.p. 125–126°, $[\alpha]_D$ −54°). If a similar relationship exists in the *p*-hydroxymandelonitrile series then, by analogy, taxiphyllin pentaacetate (m.p. 144–144·8°, $[\alpha]_D$ −22·1°) would have the D-configuration at the asymmetric centre of the aglycon and the corresponding derivative of dhurrin (m.p. 132–132·5°, $[\alpha]_D$ −50·5°) would have the L-configuration. Furthermore, only if the above

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[14] C. H. Eugster and P. Bosshard, *Helv. Chim. Acta* **46**, 837 (1963).

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configurations are assumed are the molar rotations of the D-isomers ($[M]_D$ $-11{,}313 \pm 201$) and L-isomers ($[M]_D$ $-25{,}655 \pm 655$) in the two series in excellent agreement. It seems reasonable, therefore, to assign the D-configuration to the asymmetric centre of the aglycon of taxiphyllin and the L-configuration to that of dhurrin since the presence of a substituent in the 4-position of the mandelonitrile moiety would not be expected to alter the molar rotation significantly. Evidence of this nature, however, can be misleading so it was decided to check our conclusions by PMR. Lemieux *et al.*[17] have already pointed out that the fine structure of the PMR signals for the protons of the sugar moiety of a glycoside will only alter if the aglycon changes the conformation of the sugar residue. Furthermore, the chemical shift of these protons remain relatively constant for a variety of aglycons. Consequently, the PMR spectra of the acetylated β-D-glucosides of D-mandelonitrile (prunasin tetraacetate) and D-*p*-hydroxymandelonitrile (taxiphyllin pentaacetate) would be expected to be almost identical, and differ from those containing the corresponding aglycons with the L-configuration, since the aglycons would affect the conformation of the pyranose ring in the same manner, and any long range shielding effects due to substituents in the aglycons would be very similar. Indeed, the PMR spectra of prunasin tetraacetate should only differ from that of taxiphyllin pentaacetate by the absence of an aromatic acetoxyl group at a τ value of 7·68 (Fig. 1), and the presence of a signal for five aromatic protons instead of the A_2B_2 multiplet present in the spectra of the taxiphyllin derivative. A similar argument could be applied to the corresponding derivatives of sambunigrin and dhurrin. An inspection of Fig. 1 shows that the pertinent features of the spectrum of prunasin tetraacetate are in excellent agreement with those of the corresponding derivative of taxiphyllin, and differ in the same manner from those of dhurrin pentaacetate. There seems little doubt, therefore, that taxiphyllin is β-D-glucopyranosyloxy-D-*p*-hydroxymandelonitrile, and dhurrin is β-D-glucopyranosyloxy-L-*p*-hydroxymandelonitrile. Unfortunately it has not been possible to obtain a sample of sambunigrin in order to compare its spectrum with that of dhurrin. However, we predict that the PMR spectra of these compounds will also lead to the same conclusions.

A comparison of molecular models of taxiphyllin and dhurrin pentaacetates suggests that the most stable conformation of the compound having the aglycon with the L-configuration (dhurrin) would result in the —C≡N group and the anomeric hydrogen of the glucose moiety being almost eclipsed (Fig. 2). By analogy with acetylenic compounds[12c] the long range paramagnetic shift associated with the carbon nitrogen triple bond would chemically shift the anomeric hydrogen downfield. Moreover, since the presence of the substituent on carbon 2 of the glucose moiety restricts the free rotation of the aromatic ring the latter will tend to remain in the same plane as the pyranose ring of the glucose moiety. Consequently, the carbon-hydrogen bond of the methine hydrogen of the aglycon will remain for a longer period in the same plane as the aromatic ring and the methine hydrogen will experience almost the maximum long range paramagnetic shift due to the ring current effect[12d] of the aromatic ring. On the other hand, the compound having the aglycon with the D-configuration (taxiphyllin) would not be expected to exhibit these anisotropic effects to the same extent since the most stable conformation for this compound would appear to be the one in which the carbon hydrogen bond of the methine hydrogen is

[17] R. U. Lemieux and M. Hoffer, *Canad. J. Chem.* **39**, 110–115 (1961).

normal to the plane of the aromatic ring, and almost eclipsed with the anomeric hydrogen of glucose (Fig. 2). In this case the —C≡N group lies almost in the same plane as the pyranose ring of glucose. Consequently, the methine hydrogen of the aglycon will experience a smaller paramagnetic shift due to the aromatic ring, and the anomeric hydrogen of glucose should appear at higher field since it will not experience a paramagnetic shift due to the carbon nitrogen triple bond. Indeed, these considerations account very well for the differences in the PMR spectra of the two compounds, and originally enabled us to predict the differences to be expected in the PMR spectra of the two diastereoisomers.

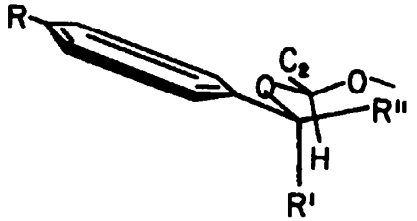

Fig. 2. The conformations of groups or atoms attached to the asymmetric carbon atom of the *p*-hydroxymandelonitrile moiety of taxiphyllin (R = OAc; R′ = H; R″ = CN) or dhurrin (R = OAc; R′ = CN; R″ = H) and carbon 1 of the glucose residue.

EXPERIMENTAL

The isolation of taxiphyllin from Taxus canadensis. A hot ethanolic extract (11.) of fresh leaves of *Taxus* (100 g) was evaporated and the residue dissolved in boiling water and filtered through Celite. The cooled filtrate was transferred to a column of coconut charcoal (100 g) and eluted successively with water (500 ml), 10% ethanol (500 ml), 50% ethanol (500 ml), absolute ethanol (500 ml) and finally with a 1:1 ethanol:benzene solution (1 l.). The coconut charcoal (Fisher Scientific Co.) had previously been washed with 2N HCl, and then with distilled water until the eluant was acid free. Removal of the solvent from the ethanol:benzene solution yielded an orange gum as a residue. The latter crystallized from ethanol:benzene as white crystalline needles (180 mg) which darkened at 163° and melted with decomposition at 168–169°, and had an $[\alpha]_D^{20}$ −66·7° (*c*, 0·372 in ethanol). (Found: C, 54·02; H, 6·54; N, 3·93%. Calc. for $C_{14}H_{17}O_7N{\cdot}C_2H_5OH$: C, 53·77; H, 6·49; N, 3·92%).

Isolation of dhurrin from Sorghum vulgare. The residue from a hot ethanolic solution (1 l.) of 6 day old seedlings (187 g) of *Sorghum vulgare* was extracted with boiling water (150 ml) and filtered through Celite. The filtrate was extracted continuously for 12 hr with ethyl acetate and the extract was evaporated to give a residue which was extracted with boiling water (150 ml). This aqueous solution was reduced to a small volume and chromatographed on silica gel using three 8″ × 8″ thin layer chromatography plates and methylethylketone:ethylacetate:formic acid: water (5:3:2:1) as irrigating solvent. Dhurrin (R_f 0·7) was located by spraying a small portion of each chromatogram with 1% $KMnO_4$ in N H_2SO_4, and isolated by scraping the appropriate band from each plate and extracting with ethanol. Removal of the ethanol under red. press. yielded dhurrin (184 mg) as a clear gum $[\alpha]_D^{20}$ −62·7° (*c*, 0·485 in ethanol), which could not be crystallized.

Properties of taxiphyllin and dhurrin. The UV absorption spectrum of taxiphyllin showed a major peak at 228 mμ (Σ_{max} 9870) and a minor peak at 272 mμ. Dhurrin gave an identical spectrum except that the Σ_{max} at 228 mμ was 13,000.

When taxiphyllin or dhurrin was hydrolysed by either 2N HCl on a steam bath, or by emulsin, glucose and *p*-hydroxybenzaldehyde were obtained in quantitative yield. The latter was isolated by crystallization from an ethereal extract of the reaction mixture. It showed no depression in m.p. when admixed with an authentic sample. Glucose was identified by thin layer chromatography[18] and paper chromatography.[19] Emulsin hydrolysis of either compound in a glass stoppered bottle containing picric acid paper indicated the release of hydrogen cyanide.

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Taxiphyllin and dhurrin pentaacetates. Acetylation of taxiphyllin with acetic anhydride in pyridine at room temperature overnight, gave a quantitative yield of a compound which crystallized from ethyl acetate–pet. ether as long white needles, m.p. 144–144·8°, $[\alpha]_D^{20}$ −22·1 (*c*, 0·320 in ethanol). (Found: C, 55·3; H, 5·38; N, 2·65. Calc. for $C_{14}H_{19}O_7N{\cdot}5(CH_2CO)$: C, 55·27; H, 5·18; N, 2·68%).

A similar reaction with dhurrin gave a product which crystallized from ethyl acetate–pet. ether as long white needles, m.p. 132–132·5°, $[\alpha]_D^{20}$ −50·5° (*c*, 0·235 in ethanol). (Found for $C_{14}H_{19}O_7N{\cdot}5(CH_2CO)$: C, 55·0; H, 5·35; N, 2·66%). The m.p. of this compound was not depressed when admixed with the pentaacetate prepared from a crystalline sample of dhurrin (m.p. 165°) which was obtained from Dr. L. Anderson.

The PMR spectra of all compounds were taken on a Varian A60 spectrometer.

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