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The Occurrence of Phenylpyruvic Acid in Woody Plants: Biosynthetic Significance

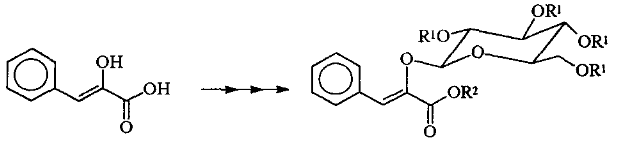
Charlene Marais, Jacobus A. Steenkamp* and Daneel Ferreira*

Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa

Abstract: The leaves and stems of *Aspalathus linearis*, a member of the Fabaceae, contains *(Z)*-3-phenyl-2-β-D-glucopyranosyloxypropenoic acid, an enolic glucoside of phenylpyruvic acid which is of relevance to the shikimic acid pathway. Copyright © 1996 Elsevier Science Ltd

Phenylpyruvic acid (PPA) features as an intermediate in the shikimic acid pathway for the biosynthesis of the crucial aromatic amino acids, L-phenylalanine and L-tyrosine in plants and bacteria[1,2]. Although PPA occurs abundantly in the urine of patients suffering from the congenital biochemical disorder phenylketonuria[3], and is effectively produced by several micro-organisms[4], certain marine sponges[5], and presumably also in tobacco plants[6], unequivocal proof for its presence in woody plants has not yet been documented. Continued investigation[7] of the physiologically significant products of secondary metabolism in *Aspalathus linearis* which is used for the manufacture of Rooibos Tea, an important health beverage, has revealed the presence of an enolic β-D-glucopyranoside of PPA hence giving credence to its role in the biosynthesis of aromatic amino acids and a variety of other metabolites in higher plants.

In addition to the phenolic compounds described previously[7], the aqueous extract of *A. linearis* contains *(Z)*-3-phenyl-2-β-D-glucopyranosyloxypropenoic acid (1) which was identified as the methyl ether acetate derivative (2) (Found: M^+, 508.1579. $C_{24}H_{28}O_{12}$ requires M, 508.1581). Its ^{1}H NMR spectrum in $(CD_3)_2CO/D_2O$ indicated an unsubstituted phenyl ring, a vinylic proton (δ 7.03, s), an *O*-methyl-(δ 3.83) and four *O*-acetyl-(δ 2.01, 1.98, 1.96 and 1.90) resonances, and the characteristic seven-spin system of the protons of a β-D-glucopyranosyl moiety ($^3J_{1'',2''} = 8.0$, $^3J_{2'',3''} = {}^3J_{3'',4''} = 9.5$ and $^3J_{4'',5''} = 10.0$ Hz) substituted at one of its oxygen functionalities. A COLOC experiment at 500 MHz correlated the methoxy protons with a carbonyl carbon (δc 164.51), the proton (δ 5.56) at the anomeric carbon (δc 99.69) with a vinylic carbon (δc 141.39) attached to oxygen, and the remaining carbon (δc 126.09) of the double bond with 2′- and 6′-H (δ 7.86, m) of the phenyl ring. When taken in conjunction with the NOE association (1%) of the methoxy protons with the vinylic hydrogen indicating a *Z*-configuration for the double bond, these data are reminiscent of structure (2) for the natural product derivative.



PPA

(1) $R^1=R^2=H$
(2) $R^1=Ac, R^2=Me$

The aforementioned tentative structure and especially the absolute configuration of the glucosidic unit were confirmed *via* synthesis of derivative (2) with PPA[8] and 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose[9] as starting materials. Thus, esterification (Cs_2CO_3MeI)[10] of PPA, followed by deprotonation of the methylphenylpyruvate (NaH, 0°C) and subsequent addition of the enolate to 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose, yielded the methyl 2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-3-phenylpropenoate (2, 21%) with ^1H NMR data identical to those of the same derivative of the natural product. Confirmation for the (*Z*)-configuration of the double bond was obtained by photolytic conversion of compound (2) in low yield into the (*E*)-geometrical isomer which showed the characteristic[11] shielding of the vinylic proton (δ 6.76) compared to its chemical shift (δ 7.03) in the *Z*-isomer (2).

(Z)-3-phenyl-2-β-D-glucopyranosyloxypropenoic acid (1) not only represents the first glucoside of PPA but also provides unambiguous evidence for the occurrence of this secondary metabolite in woody plants where it may serve as the precursor to α-hydroxychalcones and hence to C-3 oxygenated flavonoids[12]. Formation of the enolic glucoside presumably stabilizes PPA which is then 'stored' in this state and released into the biogenetic pool when required. Since biosynthetic processes are often compartmentalized the PPA glucoside may plausibly represent the form that permits intercompartmental transport.

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