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SHORT COMMUNICATION

FLAVONOIDS FROM *PHASEOLUS ATROPURPUREUS*

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Abstract—Two glycosides of kaempferol have been isolated from the leaves of *Phaseolus atropurpureus*. Their structures have been shown to be most probably kaempferol-7-L-rhamnofuranoside and kaempferol-7-(0-L-rhamnofuranosyl-(1 →5)-L-rhamnofuranoside)-3-L-rhamnofuranoside in which the major glycosidic linkages are α.

INTRODUCTION

SIRATRO is a cultivar of *Phaseolus atropurpureus* which has been developed in this laboratory for use in improved pastures for sub-tropical regions.[1] During investigations into the carbohydrates of this plant, two glycosides, of kaempferol and L(+) rhamnose, were isolated from the leaves. Preliminary analysis showed one compound to be a monorhamnoside and the other to be a trirhamnoside. The former substance did not have the same physical constants as afzelin (kaempferol-3-rhamnoside).[2] Kaempferol-7-rhamnoside, found to be the most probable structure, has been reported to occur naturally in *Lilium regale* petals.[3] It has also been isolated from the partial hydrolysate of robinin (kaempferol-7-rhamnosido-3-galactorhamnoside).[4]

RESULTS AND DISCUSSION

Three flavonoids (I–III) were isolated and purified via ion exchange resin, paper chromatography and fractional crystallization. Some of their chromatographic properties are listed in Table 1.

TABLE 1. CHROMATOGRAPHIC R_f AND M_G VALUES OF THE THREE ISOLATED FLAVONOID FRACTIONS

Flavonoid fraction	TLC		Descending paper chromatography		Ionophoresis
	Solvent*		Solvent*		0·05M Sodium borate
	A	D	A	C	(M_G)
I	0·51	0·41	0·45	0·55	0·55
II	0·66	0·58	0·83	0·82	0·18
III	0·81	0·81	0·92	0·92	0·16

* (A) ethyl acetate–pyridine–water (8:2:1); (B) ethyl acetate–acetic acid– formic acid–water (18:3:1:4); (C) *n*-butanol–acetic acid–water (4:1:5); (D) ethyl methyl ketone–water (10:1).

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[3] S. ASEN and S. L. EMSWELLER, *Proc. Am. Soc. hort, Sci.* **81**, 530 (1962).
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For TLC on silica gel, solvent D was particularly suitable giving excellent separations combined with discrete spots. Qualitative examination[5] established the presence of sugar residues in I and II but not in III. The UV spectrum of III was identical to that of kaempferol. Confirmation of the structure was obtained through the PMR[6] and IR spectra and X-ray powder photograph. These were identical to authentic kaempferol analysed in the same way.

Hydrolysis of I and II liberated the same aglycones identical to kaempferol in all the properties measured above. Only one sugar was present in both hydrolysates, and this was characterized as L(+)-rhamnose by chromatography and the preparation of its phenylosazone. Quantitative analysis[5] showed that I comprised 1 mole aglycone to 3 moles rhamnose, and II 1 mole aglycone to 1 mole rhamnose. Elemental analysis supported this evidence. The positions of attachment of the sugar residues to the aglycone were determined from consideration of the spectral evidence. UV analysis showed I to have sugar residues attached to the 3- and 7-positions of the aglycone. Similarly, II has the sugar residue at the 7-position.

The ring structure of the sugar residues was deduced through consideration of the IR spectra of the glycosides.[7] Both I and II had two peaks in the region 1010–1100 cm^{-1} apart from any attributable to the aglycone. This indicated furanoside structures for the rhamnose residues, since pyranosides are reported to give three intense peaks in this region.

The large negative specific rotation for I suggests the interglycosidic linkage is primarily the α-L-configuration. However, the PMR spectrum shows two sugar C6-methyl signals broadened, suggesting the presence of both α- and β-glycosidic linkages. II gave a specific rotation of −147°. Kaempferol-7-rhamnofuranoside has been reported isolated in both α- and β-forms from the partial acid hydrolysis of robinin.[8] The β-form had $[\alpha]^{20}$ −38·2°, and the α-form had $[\alpha]^{20}$ −200°. Hence II is probably a mixture of α- and β- forms with the α-linkage predominating. The PMR spectrum showed the presence of two split methyl signals, again suggesting a mixture of α- and β-glycosides.

Oxidation of I with hydrogen peroxide[9] liberated rhamnose. This would indicate a single rhamnose unit attached to the 3-position. Therefore, the 7-position must have a rhamnosedisaccharide attached. Robinin treated under the same conditions liberated robinobiose as expected.

The disaccaride linkage in I is tentatively suggested to be (1→5) based on evidence from ionophoresis and periodate oxidation.[10] The appreciable difference in M_G values between I and II suggests additional *cis*-hydroxyl groups available for complexing with borate. Any other linkage between two rhamnofuranoside rings eliminates one complexing site from the three available in the suggested structure for the glycoside. In addition, periodate oxidation showed that 2·52 moles periodate were reduced per mole glycoside. Theoretical reduction should be 3·0 moles periodate. Periodate resistant (1→4) linked mannose residues have been reported, and explanations based on stereochemical considerations postulated.[11] Rhamnose, having a similar C2–C3 *cis*-hydroxyl arrangement, may be subject to similar

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argument. Even so, only the postulated linkage would give a reduction of periodate greater than 2 moles per mole glycoside.

The probable structures of I and II are as shown in Fig. 1.

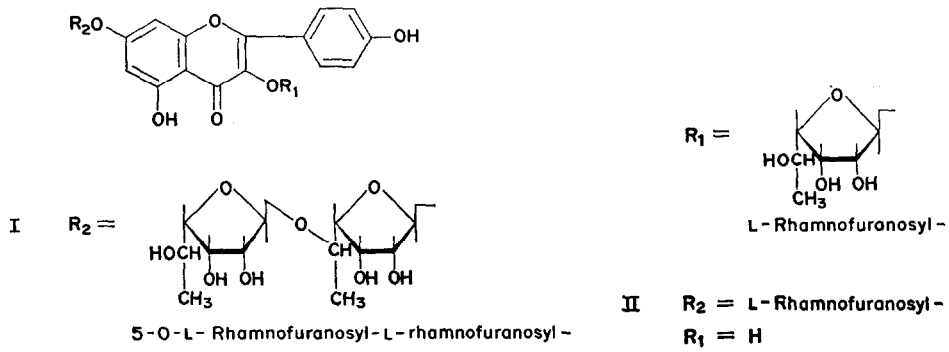

FIG. 1. PROPOSED STRUCTURES FOR I AND II.

EXPERIMENTAL

General Experimental Conditions

Phaseolus atropurpureus was grown under field conditions at the Samford Research Station, Queensland. UV spectra were obtained using a Beckmann DBG spectrophotometer, IR spectra were determined on a Unicam SP200, and PMR spectra on a Varian HA100 using dimethylsulphoxide as solvent and tetramethylsilane as internal standard. Descending paper chromatography was carried out on Whatman No. 1 paper at 25°. TLC was performed on Kieselgel layers. Sugars were detected by spraying with aniline oxalate (saturated in methanol) followed by heating at 120° for 3 min. Ionophoresis was performed in 0·05M sodium borate at 1500 V for 1·5 hr.

Flavonoids were detected under UV light by fluorescence absorbance. Concentration of solutions was under reduced pressure at 40° and melting points are uncorrected. Carbohydrate was quantitatively estimated by the phenol–sulphuric acid method.[5]

Isolation of the Flavonoid Glycosides

Extraction. Siratro leaves (100 g dry weight) were exhaustively extracted with boiling 80% EtOH. After filtration, the extract was concentrated to dryness and the residue treated with several portions of Et_2O to remove chlorophylls. TLC showed the presence of three main fluorescent compounds. The brown residue was taken up as far as possible in hot H_2O, cooled, and applied to the top of a column of Dowex AG50 cation exchange resin (H^+ form). After elution with H_2O till no carbohydrate could be detected, the column was irrigated with a gradient system of $H_2O \rightarrow$EtOH. When further carbohydrate began to be eluted, samples of eluate were removed from time to time, analysed by TLC (solvent A) and inspected under UV light. The eluates gave three fractions, each having one of the flavonoids as its main constituent.

Purification. I: On standing at 2° for 24 hr a pale-yellow precipitate was deposited. Further purification was effected on Whatman 3MM chromatography paper (solvent A). Two recrystallizations from EtOH gave a chromatographically pure compound, m.p. 201–203°, $[\alpha]^{25}$ −262° (c 0·46 EtOH). A portion crystallized from H_2O to give a chromatographically identical compound, but with a m.p. 185–187° $[\alpha]^{25}$ −266° (c 0·21 EtOH). I gave a green colour with $FeCl_3$, and the dark-brown fluorescence under UV light changed to orange on exposure to NH_3 vapour.

II: The solution containing II was concentrated to dryness and extracted several times with Et_2O. After filtration and concentration to ca. 50 ml, a yellow precipitate was deposited on storing at 2° overnight. The crystals were filtered, dissolved in a minimum quantity of warm MeOH and cooled at 2° for 24 hr. Yellow clusters were deposited, which after filtration, washing with cold MeOH, and drying, had m.p. 222–224°, $[\alpha]^{25}$ −147° (c 1·04, EtOH). II gave a green colour with $FeCl_3$, a dull yellow fluorescence with UV light, changing to bright yellow on exposure to NH_3 vapour.

III: The pure compound was isolated via Whatman 3 MM chromatography paper (Solvent A). After recrystallization from 50% EtOH III had m.p. 272–276°. A negative test for carbohydrate showed that the compound was non-glycosidic, with $FeCl_3$ it gave a green colour and it dissolved readily in 1·0N NaOH.

UV spectral analysis gave: λ_{max} (MeOH) 266, 366; ($AlCl_3$/MeOH) 270, 353, 426; (NaOAc/MeOH)

275, 383; (0·002M NaOEt) 281, 420 nm. PMR signals at $\delta = 8{\cdot}09$ (doublets, $J = 9$ c/s, H-2′, H-6′), 6·99 (doublet, $J = 9$ c/s, H-3′, H-5′), 6·50 (H-8), and 6·25 (H-6) ppm.

IR absorption: ν_{max} 1652, 1618, 1515, 1385, 1315, 1310, 1255, 1223, 1175, 1090, 1015, 981 and 892 cm^{-1}.

X-ray analysis. A photograph was obtained from powdered material on a 5·73 cm dia. camera after exposure for 30 min.

Characterization of the Glycosides

I: *Hydrolysis.* Glycoside I was heated in a sealed tube with 1·0N H_2SO_4 for 3 hr at 100°. An insoluble yellow precipitate (aglycone) was removed by filtration and the solution made up to 100 ml with water. After two recrystallizations from 50% EtOH, the aglycone was shown to be identical to kaempferol. Chromatographic analysis of the solution showed only one sugar to be present. The compound had R_g values of 3·30 (solvent A), 2·50 (solvent B), and M_G (0·05M sodium borate) 0·52. L(+) Rhamnose, run as a control, had identical chromatographic mobilities. The hydrolysate sugar and L(+) Rhamnose both gave a brick-red colour when sprayed with vanillin-perchloric acid reagent.[12] The unknown compound gave a phenylosazone, m.p. 181° undepressed when mixed with authentic L(+) rhamnose phenylosazone. The two osazones also had identical X-ray powder photographs.

Quantitative estimation of L(+) rhamnose.[5] Aliquots (0·05 ml) were removed from the hydrolysate solution, and on analysis the total rhamnose content was shown to be 140·0 mg per 207 mg (theory; 140·7) (Found: C, 54·5; H, 5·60. $C_{33}H_{40}O_{18}$ required: C, 54·7; H, 5·2%.)

Spectral data. UV spectrum of glycoside I: λ_{max} (MeOH) 266, 345; ($AlCl_3$/MeOH) 275, 349, 400; (NaOAc/MeOH) 264, 363; (0·002M NaOEt) 269, 395 nm. PMR signals at $\delta = 7{\cdot}87$ (doublet, $J = 9$ c/s, H-2′, H-6′), 6·98 (doublet, $J = 9$ c/s, H-3′, H-5′), 6·83 (doublet J = 2 c/s, H-8), 6·50 (doublet, J = 2 c/s, H-6) 5·59 and 5·36 (H-Cl rhamnose), 1·16 and 0·86 (CH_3–C6 rhamnose) ppm. IR absorption: ν_{max} 1650, 1600, 1495, 1355, 1205, 1175. 1090, 1062, 1030, 980, 848 and 821 cm^{-1}.

Oxidation with sodium metaperiodate.[10] Glycoside (46·0 mg) was suspended in aqueous $NaIO_4$ (80·9 mg in 25 ml) and shaken in the dark at R.T. Aliquots (0·25 ml) were removed at intervals, and residual IO_4 was measured after dilution to 50 ml by observing the absorbance of the solution at 223 nm. Total periodate reduced after 239 hr correspond to 2·52 moles per mole glycoside.

Oxidation with hydrogen peroxide. Glycoside (5 mg) was oxidized according to the method of Chandler *et al.*[9] Robinin (5 mg) was similarly treated in a parallel experiment. The solutions were examined chromatographically (solvent B). II: Hydrolysis was carried out as for I. The aglycone was again shown to have identical properties with kaempferol, and again the only sugar released was L(+)rhamnose. Quantitative estimation of rhamnose corresponded to 1 mole of sugar per mole aglycone. (Found: C, 58·6; H, 4·81. $C_{21}H_{20}O_{10}$ required: C, 58·3; H, 4·63%.)

Spectral data. UV spectrum of glycoside II: λ_{max} (MeOH) 265, 366; ($AlCl_3$/MeOH) 267, 354, 426; (MeOH/NaOAc) 261, 387; (0·002M NaOEt) 246, 268, 430 nm. PMR signals at $\delta = 8{\cdot}16$ (doublet, $J = 9$ c/s, H-2′, H-6′), 7·02 (doublet, $J = 9$ c/s, H-3′, H-5′), 6·86 (doublet, $J = 2$ c/s, H-8), 6·48 (doublet, $J = 2$ c/s, H-6), 5·61 (H-Cl rhamnose), 1·19 and 1·07 (CH_3–C_6 rhamnose) ppm. IR absorption: ν_{max} 1650, 1592, 1560, 1502, 1425, 1360, 1320, 1255, 1222, 1180, 1170, 1135, 1090, 1062, 1030, 975, 922, 892, 848 and 820 cm^{-1}.

Acknowledgements—The author thanks Dr. S. R. Johns for the PMR spectra and for his helpful comments and Miss R. Baker for the X-ray powder photographs.

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