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SHORT COMMUNICATION

ISOLATION AND IDENTIFICATION OF TWO ISOMERIC NARINGENIN RHAMNODIGLUCOSIDES FROM GRAPEFRUIT

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Abstract—Two isomeric rhamnodiglucosides of naringenin have been isolated from the segments of Texas Ruby Red grapefruit and identified as 4′β-D-glucosyl-7β-neohesperidosylnaringenin and 4′β-D-glucosyl-7β-rutinosylnaringenin.

INTRODUCTION

THE flavanone glycoside naringin, which is the 7β-neohesperidoside of naringenin (4′,5,7-trihydroxyflavanone), has long been known as a principal bitter constituent of the grapefruit, *Citrus paradisi*. Recently this fruit has been shown also to contain significant quantities of a tasteless isomer of naringin, naringenin-7β-rutinoside,[1] as well as lesser amounts of the 7β-neohesperidosides of isosakuranetin (5,7-dihydroxy-4′-methoxyflavanone)[2] and hesperetin (3′,5,7-trihydroxy-4′-methoxyflavanone)[3] and their rutinoside isomers.[1] All six of these glycosides are diglycosides, being derivatives of the disaccharides neohesperidose (2-*O*-α-L-rhamnopyranosyl-D-glucose)[4] and rutinose (6-*O*-α-L-rhamnopyranosyl-D-glucose).[5,6]

RESULTS AND DISCUSSION

We have now isolated two flavanone triglycosides from grapefruit and have identified them as 4′β-D-glucosyl-7β-neohesperidosylnaringenin (4′β-D-glucoside of naringin) and 4′β-D-glucosyl-7β-rutinosylnaringenin (4′β-D-glucoside of naringenin-7β-rutinoside). These compounds were purified by column chromatography. Acid hydrolysis, determination of sugar-aglycone ratios, and thin-layer chromatography established that they are naringenin rhamnodiglucosides, and their structures were confirmed by spectrophotometry, enzymatic hydrolysis, and thin-layer chromatography. The 4′-glucoside of naringenin-7β-rutinoside is tasteless; the 4′-glucoside of naringin was not available in sufficient quantity for unequivocal taste evaluation, but limited studies indicate that if this compound is bitter at all, it is probably much less so than naringin.

To our knowledge, this is the first report of the isolation of flavanone triglycosides from

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any source. The naringenin triglycosides isolated in this study are much more water soluble than the corresponding diglycosides, and are of particular interest because of their possible importance in the metabolism of naringin and naringenin-7β-rutinoside in grapefruit. Thin-layer chromatography studies indicate that 4′β-D-glucosyl-7-neohesperidosylnaringenin and 4′β-D-glucosyl-7-rutinosylnaringenin are probably also constituents respectively of the shaddock (*C. grandis*) and the sweet orange (*C. sinensis*).[7]

EXPERIMENTAL

Segments from 25 lb of Ruby Red grapefruit obtained near Weslaco, Texas, in early April, 1964, were thoroughly extracted with methanol, and the extract was concentrated *in vacuo* to a volume of 1 l. Individual compounds were isolated from this extract and purified by column chromatography procedures similar to those previously described.[1] The extract was chromatographed on a 64 × 8 cm column of Polyclar AT polyvinylpyrrolidone (General Aniline and Film Corp., Grasselli, N.J.) and eluted with distilled water. A 3 l. fraction, moving off the column after 6 l. of water had passed through, was reduced to small volume and diluted with 2-propanol; the precipitate thus obtained (1·66 g) was chromatographed on a 50 × 7·5 cm column of Polyclar AT in 25% methanol–75% benzene. The column was eluted with 22 l. of 25% methanol–75% benzene, and the methanol concentration was then increased 5 per cent for every 4 l. of solvent. A 4 l. fraction, called C-14, which was eluted after 45 l. of solvent had passed through the column, and the 4 l. fraction, C-15, which was eluted immediately following C-14, were shown by thin-layer chromatography to contain two closely related flavonoid compounds not previously identified in grapefruit. Fraction C-14 was further purified by additional chromatography on a 2·5 × 20 cm Polyclar AT column and a 1·2 × 8 cm silicic acid column, with 70% benzene–30% methanol and 75% benzene–25% methanol, respectively, as solvents; 39·7 mg of purified C-14 were obtained. C-15 was subjected to further chromatography on a 3 × 15 cm Polyclar AT column with 75% benzene–25% methanol as solvent, and on a 1·5 × 10 cm column packed with Woelm polyamide (column grade, Alupharm Chemicals, New Orleans, La.) and eluted with nitromethane containing from 5 to 15% methanol; 8·2 mg of purified C-15 were obtained.

The appearance on TLC and the u.v. spectra (λ_{max} 283 and 330 nm) of both C-14 and C-15 were typical of flavanone glycosides, but the R_f values of these compounds did not match those of any flavanone glycoside previously isolated from grapefruit (Table 1). Both compounds were found, by acid hydrolysis and examination of the hydrolysis products by TLC[1], to consist only of naringenin, rhamnose, and glucose.

TABLE 1. R_f VALUES OF REFERENCE COMPOUNDS COMPARED WITH THOSE OF C-14 AND C-15 BEFORE AND AFTER HYDROLYSIS WITH GLUCOSIDASE

Compound	R_f on given thin-layer in solvent system[1]				
	Woelm Polyamide		Avicel SF		
	1	2	3	4	5
C-14	0·27	0·85	0·08	0·29	0·02
C-15	0·22	0·81	0·08	0·31	0·03
Naringenin-7β Rutinoside	0·37	0·62	0·79	0·56	0·32
Hydrolyzed C-14	0·37	0·62	0·79	0·56	0·32
Naringin	0·30	0·60	0·82	0·60	0·38
Hydrolyzed C-15	0·30	0·60	0·82	0·60	0·38
Neohesperidin	0·43	0·61	0·68	0·57	0·35
Hesperidin	0·50	0·63	0·66	0·52	0·29
Poncirin	0·60	0·62	0·55	0·72	0·56
Isosakuranetin-7β-Rutinoside	0·67	0·63	0·52	0·67	0·53

Solvent systems: (1) nitromethane-methanol (5:2, v/v); (2) methanol-water (1:1, v/v); (3) benzene-ethyl acetate-formic acid-water (18:42:12:5, v/v/v/v); (4) 1-butanol-acetic acid-water (6:1:2, v/v/v); (5) methyl-isobutylketone-formic acid-water (14:3:2, v/v/v).

[7] J. W. MIZELLE, Unpublished data, University of Oklahoma (1966).

The relative proportions of aglycone and sugars present in C-14 and C-15 were determined by means of chromatography-densitometry[8] and fluorescence[9] procedures recently developed in our laboratory. For each compound, the sugars and aglycone obtained by acid hydrolysis of approximately 1 mg of glycoside were quantitatively separated by passing the hydrolysate through a small aqueous Polyclar AT column. The sugars were eluted from the column with water, and were separated, after deionization, by chromatography on silica gel thin-layers with *n*-propyl alcohol-ethyl acetate-water (7:1:2, v/v/v). The individual sugars were quantitated directly on TLC by densitometry.[8] The aglycone was eluted from the Polyclar column with methanol, and was quantitatively determined by measurement of its fluorescence intensity in methanolic $AlCl_3$ solution at 525 nm when excited by 325 nm light.[9] These analyses showed that the ratio of naringenin:rhamnose:glucose in both C-14 and C-15 was 1:1:2, revealing that both compounds were naringenin rhamnodiglucosides.

The positions of sugar attachment to the aglycone naringenin in the flavanone triglycosides were determined by spectral studies. Addition of anhydrous NaOAc to an alcoholic solution of either C-14 or C-15 failed to produce a shift in the λ_{max} at 283 nm, showing that neither compound possessed a free 7-hydroxy group; the λ_{max} at 283 was shifted to 305 nm in alcoholic $AlCl_3$ in both cases, indicating the presence in both compounds of a free 5-hydroxy group.[10,11] After standing for 10 min in dil. alcoholic NaOH neither C-14 nor C-15 gave the broad maximum at 430 nm typical of flavanones possessing a blocked 7-hydroxy and a free 4′-hydroxy group, giving instead a maximum at 358 nm after 30 min; hence both compounds were indicated to have no free 4′-hydroxy group.[11] These results clearly showed that glycosidic linkages in C-14 and C-15 must be at both the 4′-hydroxy and 7-hydroxy groups of naringenin.

C-14 and C-15 were subjected to hydrolysis by a specific flavonoid β-glucosidase[12] and the products were studied by TLC and spectrophotometry. In both cases glucose and a naringenin rhamnoglucoside, shown by spectral studies to possess free 4′- and 5-hydroxy groups and a blocked 7-hydroxy group, were obtained. The naringenin rhamnoglucosides obtained respectively from C-14 and C-15 were shown to be naringenin-7β-rutinoside and naringin (Table 1). Hence C-14 was identified as 4′β-D-glucosyl-7β-rutinosylnaringenin and C-15 as 4′β-D-glucosyl-7β-neohesperidosylnaringenin.

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