Glycosides and Oligosaccharides in the L-Rhamnose Series

Part IV  Synthesis of Naringin and Neohesperidin

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Naturally occurring flavanone-7-neohesperidoside, naringin and neohesperidin contained in citrus peels were synthesized. β-Neohesperidose heptaacetate was treated with hydrogen bromide in acetic acid, giving hexaacetyl α-neohesperidosyl bromide. The latter compound coupled with phloroacetophenone in the presence of silver carbonate in quinoline yielded phloroacetophenone-4-neohesperidoside after deacetylation.

Condensation of phloroacetophenone-4-neohesperidoside with p-hydroxybenzaldehyde and isovanillin respectively in the presence of strong alkali afforded the corresponding chalcone-neohesperidosides, which were converted by ring closure to naringin and neohesperidin respectively.

Furthermore, the reactivity among phloroacetophenone-4-glycosides, namely α-D-glucoside, α-D-xyloside, and α-neohesperidoside and fifteen kinds of substituted benzaldehydes was investigated. Phloroacetophenone-4-α-D-glucoside reacted with p-hydroxybenzaldehyde, p-anisaldehyde isovanillin and protocatechualdehyde. In the case of phloroacetophenone-4-α-D-xyloside the same result was obtained except the case of protocatechualdehyde.

In the case of phloroacetophenone-4-neohesperidoside reacted only with p-hydroxybenzaldehyde and isovanillin.

INTRODUCTION

A remarkable characteristic of citrus fruits is their high constant of flavanone-glycosides, which occur principally in the peel.

Among the important and easily accessible compounds in the numerous groups are naringin, neohesperidin, poncirin and hesperidin. Hesperidin, described already as early as 1828,1) is predominant flavonoid in lemons and ordinary sweet oranges (citrus sinensis) and whose structure has been completely determined as hesperetin-7-β-rutinoside.

Hesperidin was synthesized by condensing 2,3,4-triacetyl-α-L-rutinosyl-bromide with hesperetin followed by deacetylation with alkali by G. Zemplén in 1943.2) Neohesperidin was originally isolated by Kolle and Gloppe in 1936.3)

Hydrolysis of this material yielded rhamnose, glucose and hesperetin. Zemplén and Tettamanti4) found that the probable difference between hesperidin and neohesperidin lies in the point of attachment of rhamnose to glucose and suggested that in neohesperidin rhamnose is bonded to C4-hydroxyl group of glucose and this new saccharide was named neohesperidose.

Naringin appears as the predominant flavonoid of the grape fruit and pummels and has been found also in the trifoliate orange in

2) G. Zemplen and R. Bognar, Ber., 76B, 773 (1943).
certain varieties of sour oranges. The main structural features of naringin had been illustrated by Asahina and Inubuse⁵¹ (1929), Rangaswami, Seshadri and Veeraraghaviah⁷⁴ (1939) respectively. Their results indicated that naringin is 7-rhamno β-glucoside of naringenin.

Furthermore poncirin was found in Pseudaege trifoliate by S. Hattori, M. Hasegawa and M. Shimokoriyama⁷ in 1944 and whose structure was shown to be 7-rhamnoglucoside of isosakuranetin.

Nakabayashi and Horowitz, independently reported that naringin, neohesperidin and poncirin all contain the same disaccharide neohesperidose because on treatment with alkali they yielded the same degradation product, phloroacetophenone-4-neohesperidoside.

The structure of neohesperidose is 2-O-α-L-rhamnosyl β-glucose as shown by methylation studies and optical rotations value according to Horowitz. But Nakabayashi considered neohesperidose to be 4-O-L-rhamnosyl β-glucose, in accordance with Zemplén’s suggestion. This discrepancy must be reinvestigated.

The authors have synthesized naringin and neohesperidin in order to determine their chemical structure synthetically and show that neohesperidose is 2-O-α-L-rhamnopyranosyl β-glucose. The synthetic route of these substances were as Fig. 2.

![Fig. 3. Syntheses of Naringin and Neohesperidin](image)

R₁=OH R₂=H Naringin
R₁=OCH₂ R₂=H Neohesperidin

β-Neohesperidose heptaacetate was treated with hydrogen bromide saturated at 0°C in acetic acid, giving hexaacetyl-α-neohesperidosyl bromide.

The latter compound condensed with phloroacetophenone and the condensation product, upon deactylation gave phloroacetophenone-4-neohesperidose. The physical and chemical properties were identified with those of the compound obtained by alkali degradation of naringin and neohesperidin.8) (Fig. 3)

Condensation of phloroacetophenone-4-neohesperidoside with p-hydroxybenzaldehyde and isovanillin respectively in the presence of strong alkali (60%) yielded corresponding chalcon-neohesperidosides in each case in orange crystals, which could be converted to naringin and neohesperidin by ring closure. Furthermore the reactivity among phloroacetophenone-4-glycosides, namely -β-D-glucoside, -β-D-xyloside, -β-neohesperidoside and 15 kinds of substituted benzaldehyde, namely benzaldehyde, o, m, p-hydroxybenzaldehyde, o, m, p-methoxybenzaldehyde, vanillin, isovanillin, protocatechualdehyde, 4-ethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, p-hydroxybenzaldehyde-4-glucoside and vanillin -4-glucoside were investigated.

Phloroacetophenone-4-β-D-glucoside reacted with p-hydroxybenzaldehyde, p-anisaldehyde, isovanillin and protocatechualdehyde affording the corresponding chalcone-glycosides. In the case of phloroacetophenone-β-D-xyloside the same result was obtained except the case of protocatechualdehyde. Phloroacetophenone-4-β-neohesperidoside reacted only with p-hydroxybenzaldehyde and isovanillin.

**EXPERIMENTAL**

1) **Paperchromatography.** Paperchromatography of flavonoid compounds were carried out as follows. Paperchromatograms of flavonoid compounds were run in the solvent system of BuOH : AcOH : H2O (4 : 1 : 2) by ascending method and detected by spraying with diazotized sulfanillic acid solution.

2) **Hexaacetyl-α-neohesperidosyl bromide.** Heptaacetyl-β-neohesperidoside was prepared according to the author's previous publication.10) Ten grams of heptaacetyl-β-neohesperidoside was rubbed with 50 ml of saturated hydrogen bromide solution of acetic acid and kept for 6 hr. at 0°C. Then the reaction mixture was poured into ice and extracted three times with chloroform.

The chloroform extracts were collected and washed three times with saturated sodium bicarbonate solution at 0°C and then three times with cold water. The chloroform phase was dried over anhydrous calcium chloride and filtered. The filtrate was concentrated to a sirup under reduced pressure.

The sirup was thought to be hexaacetyl-α-neohesperidosyl bromide but was not able to be crystallized from any solvent; yield 8 g. Anal. Found: C, 45.68; H, 5.12. Calcd. for C24H32O14Br.: C, 45.01; H, 5.04%. [α]D = −16.50° (c 0.5, CHCl3).

3) **Phloroacetophenone-4-neohesperidoside.** Three grams of anhydrous phloroacetophenone, 14.0 g of hexaacetyl α-neohesperidosyl bromide, 2.4 g of Ag2O and 20 ml of quinoline were ground together in a mortar and the mixture was kept for 3 hours and then diluted with 100 ml of acetic acid and the filtrate was added slowly to 5 l of ice and water with stirring. The amorphous precipitates were collected by filtration, washed with water several times.

The material (2.0 g) in 15 ml of absolute methanol and 15 ml of 0.2M sodium methylate solution was boiled for 3 min. and to the solution a small amount of water was added.

The solution was neutralized with resin IR 120 (H+) and filtered rapidly. On removal of the solvent the sirup which consisted of two component was obtained. A small amount of unreacted phloroacetophenone and a large amount of phloroacetophenone-4-neohesperidoside were detected by paper chromatography.

The sirup was dissolved in water and chromatographed on a column of cellulose powder and eluted by the mixture of BuOH : AcOH : H2O (4 : 1 : 1).

The fraction of phloroacetophenone-4-neohesperidoside was collected and the solvent was distilled off under reduced pressure.

The residual sirup was crystallized from hot water in fine needles, yield 0.2 g, m.p. 162°C. Its melting point showed no depression with authentic specimen which was obtained by alkali degradation of naringin.

10) S. Kamiya, S. Esaki and M. Hama, This Journal, 31, 261 (1967).
according to the procedure of Horowitz. *Anal.* Found: C, 50.38; H, 5.90. Calcd. for C_{25}H_{28}O_{18}: C, 50.42; H, 5.92%.

4) **Synthesis of naringin.**

i) Naringin-chalcone. a) One gram of phloroacetophenone-4-neohesperidoside and 0.3 g of p-hydroxy benzaldehyde were suspended in 1 ml of ethanol, to which 7 ml of 60% aqueous potassium hydroxide solution was added in portions while maintaining the temperature at 2~3°C in an ice bath.

After the addition was over, the vigorous shaking was continued for 24 hr., at room temperature. The colouration of the reaction mixture changed from slight yellow to reddish-brown and a small quantity of undissolved p-hydroxybenzaldehyde was remained.

Then 10 ml of water was added to it and cooled again in an ice and water bath and 10% hydrochloric acid solution was added with stirring until the solution was acidified to pH 2.

The resulting orange yellow precipitates were recrystallized from 50% ethanol as yellow needles; m.p. 185~200°C, undepressed on admixture with authentic specimen. Yield 0.8 g. *Anal.* Found: C, 54.16; H, 5.70. Calcd. for C_{27}H_{32}O_{14}H_{2}O: C, 54.18; H, 5.73%.

The chalcone gave a brown colour with ferric chloride and well separated as a yellow spot on a paper chromatogram, not accompanied by even a faint spot of corresponding naringin.

Its UV spectrum, Fig. 4, was identical with that of the chalcone form of naringin.

b) Naringin-chalcone was also prepared in order to use as authentic specimen according to the Shimokoriyama’s procedure.

ii) Naringin-chalcone acetate. a) Naringin-chalcone acetate was prepared and used as the authentic specimen.

To a suspension of 2 g of anhydrous sodium acetate in 15 ml of acetic anhydride was added 2 g of natural naringin and the mixture was boiled in an oil bath under reflux for five hours.

On cooling the solution was poured into ice water allowed to stand in a refrigerator overnight.

The material precipitated was collected by filtration

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**FIG. 4.**

- - - Naringin chalcone (synthetic)
- - - Naringin chalcone (from naringin)
- - - Naringin (natural)
--- Naringin (synthetic)

and washed several times with water and recrystallized from ethanol to the slight yellowish crystals; yield 1.8 g, m.p. 125°C. Anal. Found: C, 56.43; H, 5.24. Calcd. for C_{45}H_{49}O_{23}: C, 56.42; H, 5.16%.

b) Synthetic chalcone (0.3 g) described above was dissolved in the mixture of 10 ml of acetic anhydride and 10 ml of pyridine and allowed to stand overnight at 30°C. The solution was evaporated in vacuo and the residue was dissolved in ethanol and again evaporated in vacuo. This treatment was repeated three times. The residue was recrystallized from aqueous ethanol to the slight crystals yellowish; yield 0.3 g, m.p. 125°C, no depression on admixture with authentic specimen.

iii) Naringin. The chalcone (0.5 g) obtained above was heated with 0.5 ml of 50% ethanol and 0.5 ml of McIlvaine buffer solution (pH 7) for three min. in a boiling water bath until almost decolouration of the reaction mixture. The solution was added to an equal volume of water and allowed to stand overnight in a refrigerator. The crystals which separated were recrystallized from water as colourless needles, undepressed on admixture with authentic specimen; yield 0.4 g, m.p. 80–83°C, [α]_{D}^{2} = 82° (c 1, 90% Et OH).

After having been dried in the vacuum desicator for three days it melted at 172–173°C (Lit. 171°C). RF value was also indistinguishable from that of natural naringin. Both UV and IR spectra were found to be identical with those of authentic specimen (Fig. 4).

On hydrolysis with 5% sulphuric acid solution in the usual manner it yielded naringenin, rhamnose and glucose, which were identified by paperchromatography with authentic specimens.

The alcoholic solution of synthetic naringin gave a reddish colouration to HCl-Mg test. Anal. Found: C, 54.08; H, 5.62. Calcd. for C_{27}H_{32}O_{14}·H_{2}O: C, 54.18; H, 5.73%.

5) Synthesis of neohesperidin.

i) Isovanillin. Although several synthetic methods of isovanillin have been known, a new synthetic method was investigated in this paper.

Metallic sodium (0.4 g) was dissolved in 12 ml of absolute methanol and to the solution were added 2.3 g of protocatechualdehyde and 3.1 g of methyl p-toluenesulphonate and the mixture was refluxed in mixture with authentic specimen; yield 0.4 g, m.p. 80–83°C, [α]_{D}^{2} = 82° (c 1, 90% Et OH).

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5) Synthesis of neohesperidin.

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Metallic sodium (0.4 g) was dissolved in 12 ml of absolute methanol and to the solution were added 2.3 g of protocatechualdehyde and 3.1 g of methyl p-toluenesulphonate and the mixture was refluxed in
an oil bath for 2 hours at 80°C.

After the reaction was complete the precipitated sodium p-toluenesulphonate was removed and the filtrate was evaporated in vacuo.

The residue was dissolved in water and extracted three times with ether. The ethereal extracts were collected and extracted twice with 4% sodium hydroxide solution.

The extracts were collected and acidified with sulphuric acid to pH 2 and again extracted three times with ether.

The extract was washed with cold water and dried over calcium chloride. Then the filtrate was evaporated and the residual brownish product was recrystallized from hot water to slight yellowish crystals m.p. 114-115°C, undepressed on admixture with authentic specimen, yield 0.3g. Anal. Found: C, 63.32; H, 5.20. Calcd. for C8H8O3: C, 63.15; H, 5.30%.

ii) Neohesperidin-chalcone. The method of preparation and the properties of neohesperidin-chalcone have not been reported by any investigators.

a) The article compound was prepared from the corresponding flavanone neohesperidin according to the Shimokoriyama's procedure and used as the authentic specimen.

Neohesperidin (1.3g) was heated with 2 g of potassium hydroxide and 4 ml of water on a boiling water bath for 2 min.

On cooling, the reaction mixture was acidified with 20% hydrochloric acid to pH 2.

The resulting orange-yellow precipitates were recrystallized from 30% ethanol to yellow needles; m.p. 114-115°C, undepressed on admixture with authentic specimen, yield 0.3 g. Anal. Found: C, 63.32; H, 5.20. Calcd. for C8H8O3: C, 63.15; H, 5.30%.

b) One gram of phloroacetophenone-4-neohesperidoside and 0.3g of isovanillin were suspended in 1 ml of ethanol and 7 ml of 60% potassium hydroxide solution was added in portions while maintaining the temperature at 2-3° in an ice and water bath. After the addition was over, the vigorous shaking was continued for 48 hours at room temperature. The colour of reaction mixture changed from slight yellow to reddish brown and a small quantity of undissolved isovanillin was remained.

Then 10 ml of water was added to the solution and cooled again in an ice and water bath and 10% hydrochloric acid solution was added with stirring until the solution was acidified.

The resulting orange-yellow precipitates were recrystallized from 50% ethanol as crystals m.p. 100-120°C, undepressed on admixture with authentic specimen; yield 0.8 g. Anal. Found: C, 49.49; H, 5.98. Calcd. for C28H34O15.5H2O: C, 49.92; H, 6.14%. UV spectrum was shown in Fig. 5 which was identified with authentic specimen.

iii) Neohesperidin-chalcone acetate. a) Neohesperidin-chalcone acetate was prepared and used as an authentic specimen.

One g neohesperidin was acetylated with acetic acid and anhydrous sodium acetate in a similar manner as in the preparation of naringin-chalcone acetate.

Slight yellowish crystals. Yield 0.8 g m.p. 121°C.

b) Synthetic neohesperidin chalcone (0.31 g) was dissolved in a mixture of 10 ml of acetic anhydride and 10 ml of pyridin and allowed to stand overnight at 30°C.

After the solvent was removed, the residue was dissolved in ethanol and evaporated in vacuo.

After the treatment was repeated three times, the residue obtained was recrystallized from aqueous ethanol.

Yield 0.3 g m.p. 121°C. No depression was found on admixture with authentic specimen.

iv) Neohesperidin. The yellow chalcone (0.8 g) was heated with 8 ml of 50% ethanol and 1 ml of McIlvaine buffer solution (pH 6) for five min. in a boiling water bath until almost decolouration of the reaction mixture.

The solution was added to an equal volume of water and allowed to stand overnight in a refrigerator.

The crystals which separated were recrystallized from aqueous ethanol.

Yield 0.3 g m.p. 121°C. No depression was found on admixture with natural neohesperidin.

Both UV and IR spectra were found to be identical with those of authentic specimen (Fig. 5).

On hydrolysis with 5% sulphuric acid solution in the usual manner it yielded hesperetin, rhamnose and glucose.

The alcoholic specimen gave a reddish colouration to Mg-HCl test. Anal. Found: C, 55.12; H, 5.70. Calcd. for C28H34O15: C, 55.08; H, 5.61%.

v) Phloroacetophenone-4-β-D-xyloside triacetate. Phloroacetophenone (11.2 g) and 27.5 g. of triacetyl α-D-xylosylbromide were dissolved in 75 ml of acetone and to this solution 37.5 ml of sodium hydroxide solution (9 g NaOH in 100 ml water) was
added in small portions on cooling in an ice water bath.

The crystalline material was soon deposited, but on stirring mechanically for a while the material dissolved into the solution.

The resulting solution, two layers, was shaken for one hour at room temperature and again 75 ml of acetone was added in one portion, resulting in homogeneous solution of yellow colouration.

After being left to stand overnight at room temperature the solution was evaporated under reduced pressure in an water bath at 35°C and the sirupy residue was washed several times with cold water in order to remove water soluble materials. Then the sirup was dissolved in the hot mixture of 130 ml of methanol and 100 ml of water and allowed to stand for two hours at room temperature.

The colourless needles deposited were collected, washed twice with cold methanol; yield 9.5 g, m.p. 115–117°C. Anal. Found: C, 53.34; H, 5.17. Calcd. for C₁₉H₂₂O₁₁: C, 53.52; H, 5.20%

In order to determine the attachpoint of sugar residue to the aglycon, methylation experiment was carried out.

Phloroacetophenone-4-xyloside triacetate (5 g) in 40 ml of absolute methanol and 40 ml of 0.2 M sodium methylate solution was boiled for 3 min. and to the solution a small amount of water was added.

The solution was neutralized with resin IR 120(H⁺) and filtered.

A mixture of 2 g of a sirup (phloroacetophenone-4-xyloside), 12 ml of methyl iodide, 13 g of potassium carbonate and 400 ml of acetone was boiled under reflux for 2 days.

The residue obtained by filtration and evaporation of the mixture was dissolved in water and allowed to stand in an ice box.

The crystals deposited were collected, m.p. 127–128°C after recrystallization from acetone, no depression on admixture with authentic 2,6-dimethylphloroacetophenone (m.p. 128°C).

From the above experiment it was confirmed that xylose residue was attached to 4-hydroxyl group of phloroacetophenone.

vi) Phloroacetophenone-4-β-D-glucoside tetraacetate was synthesized according to the Zemplén's procedure.

ii) Reactivities between phloroacetophenone-4-β-glycosides and substituted benzaldehydes. Phloroacetophenone-4-β-D-xyloside, -4β-D-glucoside and -4-neohesperidoside were used in the experiment.

As substituted benzaldehydes β-hydroxybenzaldehyde, β-methoxybenzaldehyde, vanillin, isovanillin and protocatechuialdehyde, were used.

Phloroacetophenone-4-glycoside (1 g) and 0.3 g of substituted benzaldehydes were suspended in 1 ml of ethanol and 7 ml of 60% potassium hydroxide solution was added in portions in an ice water bath and the vigorous shaking was continued for three days.

Then 10 ml of water was added to it and the mixture cooled again in an ice water and 10% hydrochloric acid solution was added to it with stirring until the solution was acidified to pH 2.

The resulting orange or yellow precipitates were recrystallized from 50% ethanol. The results were shown in Table I.

<table>
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<th>Sugar Residue</th>
<th>Xylose</th>
<th>Glucose</th>
<th>Neohesperidoside</th>
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<tbody>
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<td>155–160°</td>
<td>185–190°</td>
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<td>orange</td>
<td>orange</td>
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<td>164°</td>
<td>—</td>
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<td>—</td>
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<tr>
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<td>125°</td>
<td>105°</td>
<td>96–98°</td>
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<tr>
<td>R₂, OH</td>
<td>—</td>
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<td>—</td>
</tr>
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</table>

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