

Sweetness of Hesperetin Dihydrochalcone Derivatives Linked to ω -Amino Acid or Its Oligomer

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Synopsis. Derivatives of hesperetin dihydrochalcone having ω -amino acid, oligoglycine, or oligo-(γ -aminobutyric acid) were synthesized and tasted. The hydrophobicity of the introduced group is important for understanding the structure-sweet taste relationship of these compounds, though other factors than hydrophobicity also must be taken into account.

Neohesperedin dihydrochalcone (**1**) is an intensely sweet substance derived from a rind-constituent of citrus fruits.¹⁾ The aglycone of **1**, namely hesperetin dihydrochalcone (**2**) (DHC-OH), and its ω -carboxyalkyl derivatives DHC-O(CH₂)_mCO₂H (**3**) ($m=1, 3$) and ω -sulfoalkyl derivatives DHC-O(CH₂)_mSO₃H (**4**) ($m=1-4$) are also known to elicit sweet sensation.²⁾ We have attempted to prepare derivatives of **2** having an amino acid or peptide in group R in Fig. 1 for the purpose of investigating the structure-sweet taste relationship of these compounds.

Results and Discussion

Oligomers of glycine were introduced to **3** ($m=3$)³⁾ affording DHC-O(CH₂)₃CO(NHCH₂CO)_nOH (**5**). Results of semiquantitative taste evaluation are given in Table 1. The compound having one glycyl residue, **5** ($n=1$), is sweeter than the parent compound **3** ($m=3$), but further addition of glycyl residue results in decrease in sweetness. Another series of derivatives prepared includes those having ω -amino acid residue

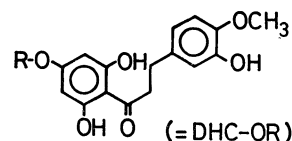


Fig. 1. Structure of hesperetin dihydrochalcone derivatives (DHC-OR).

1: R = C₁₂H₂₁O₉ (β -neohesperidosyl), **2:** R = H, **3-8:** see text and Tables 1 and 2.

in the side chain, namely DHC-O(CH₂)₃CONH-(CH₂)_nCO₂H (**6**), the sweetness of which is summarized in Table 2. Successive addition of a methylene group causes marked decrease in sweet potency, finally giving tasteless derivative **6** ($n=5$).

Since the sweetness of aglycone **2** is of the same order of magnitude as that of the parent glycoside **1**,^{2a)} group R in Fig. 1 is considered not to be directly involved in the interaction of these sweetener molecules with the taste receptors. DuBois *et al.*⁴⁾ recently reported zwitterionic amino acid derivatives of **2**, relating their relative sweetness to a chromatographic parameter which quantitates the hydrophobic-hydrophilic balance of molecule. We assumed that the change in hydrophobicity caused by the introduction of group R would mainly affect the sweet potency. Thus the structure-sweet taste relationship found here for the **5** and **6** series can be explained in terms of increase and decrease in hydrophobicity of molecule,

TABLE 1. RELATIVE SWEETNESS, MELTING POINTS, AND ANALYTICAL DATA OF DHC-O(CH₂)₃CO(NHCH₂CO)_nOH (**5**)

<i>n</i>	Relative sweetness ^{b)}	Mp θ_m /°C (crystallized from)	Found(%)			Calcd(%)		
			C	H	N	C	H	N
0 ^{a)}	300	190—192 ^{b)} (EtOH)						
1	500	126—130 (MeOH)	58.18	5.65	3.14	57.89	5.74	3.07 ^{c)}
2	400	175—176 (MeOH)	56.61	5.67	5.24	56.63	5.64	5.50 ^{d)}
3	300	110—118 (MeOH-AcOEt)	53.20	5.57	7.25	53.06	5.82	7.14 ^{e)}
4	200	200—205 (MeOH)	51.72	5.88	8.94	52.09	5.78	8.68 ^{e)}

a) Compound **3** ($m=3$). b) Ref. 3, 193—195 decomp. c) $\frac{1}{2}$ H₂O. d) $\frac{1}{4}$ H₂O. e) $\frac{3}{2}$ H₂O.

TABLE 2. RELATIVE SWEETNESS, MELTING POINTS, AND ANALYTICAL DATA OF DHC-O(CH₂)₃CONH(CH₂)_nCO₂H (**6**)

<i>n</i>	Relative sweetness ^{b)}	Mp θ_m /°C (crystallized from)	Found(%)			Calcd(%)		
			C	H	N	C	H	N
1 ^{a)}	500	see Table 1						
2	200	66—68 (lyophilized)	60.13	5.66	3.07	59.86	5.90	3.04
3	200	178—183 (MeOH)	60.20	6.14	3.03	60.62	6.15	2.95
4	100	166—167 (MeOH)	60.03	6.33	2.74	59.87	6.76	2.69 ^{b)}
5	0	204—208 decomp (MeOH-AcOEt)	61.63	6.51	2.60	62.01	6.61	2.78

a) Compound **5** ($n=1$). b) C₂₅H₃₁O₉N·CH₃OH.

respectively, suggesting that group R be required to possess optimal hydrophobicity for showing sweet taste. Indeed, maximal sweetness is developed for the compounds of the **4** series when the number of methylenes is 2.^{2b)} In the case of DHC-OCH₂CONH(CH₂)_n-CO₂H (**7**), relative sweetness⁵⁾ is as follows: 200 (*n*=1), 200–300 (*n*=2), 200 (*n*=3), and 100–200 (*n*=5), *i.e.*, the compound (*n*=2) is the sweetest in this series, though its sweetness is less than that of **5** (*n*=1). In view of substituent constants of hydrophobic parameters⁶⁾ it is expected that the hydrophobicity of a molecule will not be much affected by the introduction of a γ -aminobutyric acid residue NH(CH₂)₃CO. The sweetness of DHC-O(CH₂)₃CO[NH(CH₂)₃CO]_nOH (**8**) (100, *n*=2; 0, *n*=4)⁵⁾ is, however, less than those of the corresponding oligoglycine derivatives **5**, indicating that not only the hydrophobicity but also other factors must be considered for better understanding of the structure-sweet taste relationship of these compounds.

Experimental

Melting points were uncorrected. Compounds **3** (*m*=1 and 3) were prepared from hesperetin as described in Ref. 3.

N-Hydroxysuccinimide Ester of **3** (*m*=3). To an ice-cooled solution of 4 mmol of **3** (*m*=3) and 6 mmol of *N*-hydroxysuccinimide in tetrahydrofuran (10 cm³) was added 4.4 mmol of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and the mixture was stirred at room temperature. After 42 h the solution was evaporated and the residue dissolved in AcOEt was washed successively with aqueous solutions of 10% citric acid, 4% NaHCO₃, and saturated NaCl. The *N*-hydroxysuccinimide ester was crystallized from AcOEt-hexane, mp 137–142 °C (Found: C, 58.55; H, 5.29; N, 2.93%. C₂₄H₂₅O₁₀·1/4H₂O).

General Procedures. *Method A:* Amino acid or peptide (0.6–0.7 mmol) was dissolved in H₂O containing Et₃N (0.6–0.7 mmol) and added to a solution of 0.5 mmole of *N*-hydroxysuccinimide ester of **3** (*m*=3) in tetrahydrofuran. After stirring at room temperature the reaction mixture was acidified and evaporated and the residue was subjected to Sephadex LH-20 column chromatography using MeOH as eluent. Crystallization from MeOH or MeOH-AcOEt afforded the desired product **5** or **6**.

Method B: To a solution of hydrochloride of methyl or ethyl ester of amino acid or peptide (1.5 mmol) in CHCl₃ or H₂O were added Et₃N (1.5 mmol) and a solution of 1 mmol of *N*-hydroxysuccinimide ester of **3** (*m*=3) in tetrahydrofuran and stirred at room temperature. Usual workup and SiO₂ column chromatography (if necessary) gave the methyl or ethyl ester of the dihydrochalcone derivative. Without further purification the ester was dissolved in 0.5

M NaOH (1 M=1 mol dm⁻³) and stirred for 20 min at room temperature. The solution was acidified by addition of HCl aq and the precipitate was purified by Sephadex LH-20 column chromatography.

Method C: To an ice-cooled solution of 1 mmol of **3** (*m*=1), amino acid methyl or ethyl ester hydrochloride (1.5 mmol), Et₃N (1.5 mmol), and 1-hydroxybenzotriazole (0.5 mmol) in a mixture of tetrahydrofuran and *N,N*-dimethylformamide was added 1.5 mmol of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. After stirring for 24–48 h at room temperature the reaction mixture was worked up as usual and the product was subjected to alkaline hydrolysis as described in Method B.

Mp's and results of elemental analyses of **5** and **6** are given in Tables 1 and 2. Compounds **5** (*n*=2 and 3) and **6** (*n*=2–4) were synthesized with Method A. The following derivatives were prepared by using method B *via* ethyl ester: **5** (*n*=1 and 4); **8** (*n*=2), mp 166–167 °C (Found: C, 59.44; H, 6.32; N, 4.92%. C₂₈H₃₈O₁₀N₂·1/4H₂O); **8** (*n*=4), mp 131–134 °C. Compound **6** (*n*=5) was prepared with method B *via* methyl ester. Method C afforded the following compounds: **7** (*n*=1) *via* ethyl ester, mp 198–210 °C decomp (Found: C, 55.92; H, 5.21; N, 2.97%. C₂₀H₂₁O₉N·1/2H₂O); **7** (*n*=2) *via* methyl ester, mp 173–180 °C (Found: C, 57.10; H, 5.34; N, 2.91%. C₂₁H₂₃O₉N·1/2H₂O); **7** (*n*=3) *via* ethyl ester, mp 240–256 °C decomp (Found: C, 58.08; H, 5.63; N, 3.08%. C₂₂H₂₅O₉N·1/2H₂O); **7** (*n*=5) *via* methyl ester, mp 211–212 °C (Found: C, 50.65; H, 6.39; N, 3.01%).

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- 5) Relative sweetness is given on a molar basis taking sucrose as standard. One-mM solutions containing equimolar amount of NaOH were tested by a taste panel. Each solution was compared with 0.1–0.6 M sucrose solutions.
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