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$(CH_2)_mCO_2H$ (**3**) (m=1, 3) and ω-sulfoalkyl derivatives DHC–O$(CH_2)_mSO_3H$ (**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.

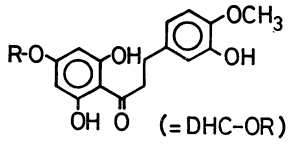

Fig. 1. Structure of hesperetin dihydrochalcone derivatives (DHC-OR).
1: R=$C_{12}H_{21}O_9$ (β-neohespesperidosyl), **2**: R=H, **3**—**8**: see text and Tables 1 and 2.

Results and Discussion

Oligomers of glycine were introduced to **3** (m=3)[3] affording DHC–O$(CH_2)_3CO(NHCH_2CO)_nOH$ (**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 in the side chain, namely DHC–O$(CH_2)_3CONH(CH_2)_nCO_2H$ (**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$(CH_2)_3CO(NHCH_2CO)_nOH$ (**5**)

n	Relative sweetness[5]	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) ½H_2O. d) ¼H_2O. e) ⅜H_2O.

Table 2. Relative sweetness, melting points, and analytical data of DHC–O$(CH_2)_3CONH(CH_2)_nCO_2H$ (**6**)

n	Relative sweetness[5]	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) $C_{25}H_{31}O_9N\cdot CH_3OH$.

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–$OCH_2CONH(CH_2)_n$-CO_2H (**7**), relative sweetness[5] 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 parameters[6] it is expected that the hydrophobicity of a molecule will not be much affected by the introduction of a γ-aminobutyric acid residue $NH(CH_2)_3CO$. The sweetness of DHC–$O(CH_2)_3CO[NH(CH_2)_3CO]_nOH$ (**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 cm^3) 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% $NaHCO_3$, 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%. $C_{24}H_{25}O_{10}\cdot\frac{1}{4}H_2O$).

General Procedures. *Method A*: Amino acid or peptide (0.6—0.7 mmol) was dissolved in H_2O containing Et_3N (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 $CHCl_3$ or H_2O were added Et_3N (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 SiO_2 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), Et_3N (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%. $C_{28}H_{36}O_{10}N_2\cdot\frac{1}{4}H_2O$); **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%. $C_{20}H_{21}O_9N\cdot\frac{1}{2}H_2O$); **7** ($n$=2) *via* methyl ester, mp 173—180 °C (Found: C, 57.10; H, 5.34; N, 2.91%. $C_{21}H_{23}O_9N\cdot\frac{1}{2}H_2O$); **7** ($n$=3) *via* ethyl ester, mp 240—256 °C decomp (Found: C, 58.08; H, 5.63; N, 3.08%. $C_{22}H_{25}O_9N\cdot\frac{1}{2}H_2O$); **7** ($n$=5) *via* methyl ester, mp 211—212 °C (Found: C, 50.65; H, 6.39; N, 3.01%).

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References

1) R. M. Horowitz and B. Gentili, *J. Agric. Food Chem.*, **17**, 696 (1969).

2) a) G. E. DuBois, G. A. Crosby, and P. Saffron, *Science*, **195**, 397 (1977); b) G. E. DuBois, G. A. Crosby, R. A. Stephenson, and R. E. Wingard, Jr., *J. Agric. Food Chem.*, **25**, 763 (1977).

3) G. E. DuBois, G. A. Crosby, and P. Saffron, *Synth. Commun.*, **7**, 49 (1977).

4) G. E. DuBois, G. A. Crosby, J. F. Lee, R. A. Stephenson, and P. C. Wang, *J. Agric. Food Chem.*, **29**, 1269 (1981).

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.

6) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).