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Chemical Structure and Sweet Taste of Isocoumarin and Related Compounds. VII[1]

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On the basis of the previous findings, relationship between the structure and sweet taste was examined by modification of the ethylene unit (B moiety) of β-(3-hydroxy-4-methoxyphenyl)ethylbenzene (**1**), by application of the theory of bioisosterism: *N*-Benzyl-3-hydroxy-4-methoxyaniline (**2**), 3-hydroxy-4-methoxybenzyl phenyl sulfide (**4**), and phenyl 3-hydroxy-4-methoxythiobenzoate (**5**) were synthesized.

The synthesis of 3-hydroxy-4-methoxybenzyl benzoate (**7**) was attempted to clarify the effect of elimination of the methylene in 4-position of 8-desoxyphyllodulcin on sweet taste.

As modification of 8-desoxyphyllodulcin, related lactone and lactam derivatives were synthesized. 1) Lactone derivatives; 2-(3-hydroxy-4-methoxyphenyl)-1,3-benzodioxan-4-one (**8**), and 2-(3-hydroxy-4-methoxyphenyl)-1,3-benzothioxan-4-one (**9**). 2) Lactam derivatives; 2(3-hydroxy-4-methoxyphenyl)-2,3-dihydroisocarbostyril (**10**), 2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-1,3-benzoxazin-4-one (**11**), 2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-1,3-benzothiazin-4-one (**12**), 2-(3-hydroxy-4-methoxyphenyl)-4-(3H)-1,2-dihydroquinazolinone (**13**), and 2-(3-hydroxy-4-methoxyphenyl)isocarbostyril (**14**).

Additionally, a lactam derivative related to phyllodulcin; 8-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-3,4-dihydroisocarbostyril (**15**), was synthesized in order to examine the effect of a hydroxyl group in 8-position on sweet taste.

The compounds **2**, **4**, **8**, and **9** had a strong sweet taste, and the compound **15** had a faint sweet taste; while the compounds **5**, **7**, **10**, **11**, **12**, **13**, and **14** were all tasteless. Based on these facts, the delicate relationship between the molecular structure and sweet taste was discussed.

Keywords——phyllodulcin; isocoumarin; sweetness; IR spectrum; structure-activity relationship; molecular modification

Our previous report[1] described the relationship between the structure and sweet taste in the modification of β-(3-hydroxy-4-methoxyphenyl)ethylbenzene (**1**),[3] which constitutes an essential structure of sweetness of phyllodulcin. The principle of the relationship between the sweet taste and the structure of **1** was as follows: Each of phenyl moiety (A moiety), ethylene moiety (B moiety), and 3-hydroxy-4-methoxyphenyl moiety (C moiety), which constitute the molecule of **1**, may be playing a part in the appearance of sweet taste, and each moiety requires some fine structural specifity to bind the sweet receptor. consequently, investigation on the molecular modification of **1** was carried out on the respective three moiety, A, B, and C.

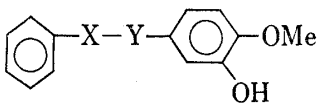
As a sequel to the previous one,[1] the present investigation was undertaken to clarify the relationship between sweet taste and structure of the B moiety. *N*-Benzyl-3-hydroxy-4-methoxyaniline (**2**), in which another methylene group of the B moiety in **1**, differing from that in 3-hydroxy-4-methoxybenzylaniline (**3**),[4] is replaced by an imino group, was synthe-

1) M. Yamato, K. Hashigaki, J. Uenishi, I. Yamakawa, N. Sato, and T. Koyama, *Chem. Pharm. Bull.* (Tokyo), **23**, 3101 (1975).
2) Location: *Tsushima-naka, 1–1–1, Okayama, 700, Japan.*
3) M. Yamato, K. Hashigaki, Y. Kuwano, and T. Koyama, *Yakugaku Zasshi*, **92**, 535 (1972).
4) M. Yamato, K. Sato, K. Hashigaki, T. Ishikawa, M. Oki, and T. Koyama, *Yakugaku Zasshi*, **94**, 359 (1974).

sized, and **2** (× 250)[5] had a strong sweet taste, similar to **3** (× 300). The results from **2** and **3** demonstrated that both methylene groups can be replaced by an imino group and they behave as bioisoster of **1**. 3-Hydroxy-4-methoxybenzyl phenyl sulfide (**4**), in which a methylene group of **1** is replaced by a sulfur atom, and phenyl 3-hydroxy-4-methoxythiobenzoate (**5**), in which another methylene group of **4** is replaced by a carbonyl group, were synthesized. The compounds **4** (× 150) had a strong sweet taste, while **5** had no sweet taste.

The reason for the loss of sweet taste in **5**, having a carbonyl group in the B moiety, may be due to the occupation of the B moiety in **5** by a double bond owing to the resonance contribution of a carbonyl group, as was considered in our previous paper.[1] Further, infrared (IR) spectra of a carbonyl group shown in Table I suggest the degree of resonance or tautomerism contribution of the B-form due to the resonance effect of hetero atoms or tautomerism effect of the methylene group, decreases in the order of imino group, methylene group, sulfur atom, and oxygen atom (Fig. 1).

Table I. UV and IR Spectra of Carbonyl Group of Related Compounds to **1**



X	Y	UV λ_{max}^{MeOH} nm (ε)	IR cm^{-1}: $\nu_{C=O}$
CH_2	CO	275(13786) 308 (9669)	1657
NH	CO	268(10265) 295 (8130)	1649
O	CO	263(14825) 297 (7925)	1703
S	CO	280(11736) 309(11480)	1660

A-form B-form

Fig. 1

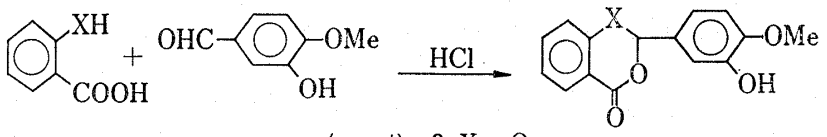
In view of sweet taste of phyllodulcin and 8-desoxyphyllodulcin (**6**)[3] having a lactone-ring, 3-hydroxy-4-methoxybenzyl benzoate (**7**), with eliminating a methylene group in 4-position from **6**, was synthesized. The compound **7** was tasteless. This fact may indicate that the conformation of the molecule was altered by the ring opening. Therefore, in order to examine the contribution of the lactone-ring to sweet taste, synthesis of lactone and lactam types was attempted.

1. Lactone Derivatives Related to 8-Desoxyphyllodulcin

In the previous modification of the B moiety in **1**, the compounds in which a methylene group in **1** was replaced by an imino group, oxygen atom, and sulfur atom, respectively, behaved as bioisoster of **1** and revealed sweet taste.[1] Based on this fact, 2-(3-hydroxy-4-

5) The sweetness of the test sample was compared with that of sucrose. An aqueous ethanol solution of sucrose containing 3% was judged to be threshold level, and the sweet level of the test sample was calculated from the concentration of the test sample which is required to attain the equivalent sweetness to that of 3% sucrose solution.

methoxyphenyl)-1,3-benzodioxan-4-one (**8**) and 2-(3-hydroxy-4-methoxyphenyl)-1,3-benzothioxan-4-one (**9**), in which a methylene group in 4-position of 8-desoxyphyllodulcin was replaced by an oxygen and a sulfur atom, respectively, were synthesized (Chart 1). Both **8** (× 100) and **9** (× 300) had a strong sweet taste, and behaved as bioisoster of **6** similarly as was found in the modification of **1**.

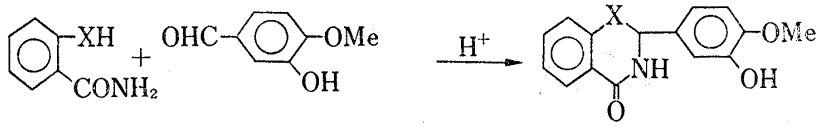


(sweet) : **8** X = O
(sweet) : **9** X = S

Chart 1

2. Lactone Derivatives Related to 8-Desoxyphyllodulcin

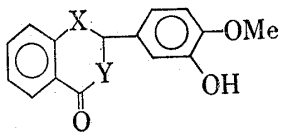
From the interesting fact that 3-hydroxy-4-methoxybenzanilide[1] is tasteless and phenyl 3-hydroxy-4-methoxybenzoate (× 1—10) has a faint sweet taste, the derivatives in which a lactone group in **6**, **8**, and **9** was replaced by a lactam group were synthesized. The compounds synthesized were 3-(3-hydroxy-4-methoxyphenyl)-3,4-dihydroisocarbostyril(**10**),[6] 2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-1,3-benzoxazin-4-one (**11**), 2-(3-hydroxy-4-methoxy-phenyl)-2,3-dihydro-1,3-benzothiazin-4-one (**12**), 2-(3-hydroxy-4-methoxyphenyl)-4-(3H)-1,2-dihydroquinazolinone (**13**),[7] and unsaturated 3-(3-hydroxy-4-methoxyphenyl)isocarbostyril(**14**)[6] (Chart 2). These lactams were all tasteless in contrast to the related sweet lactones (Table II). The difference of taste between the lactones and the lactams supports the following assumption: Owing to the resonance contribution of the forms in which a double bond occupies the central bond of the lactones and lactams, the degree of resonance effect of an imino group and an oxygen atom would differ and consequently, the spatial structure of the lactones may be differ from that of the lactams.



(tasteless) : **11** X = O
(tasteless) : **12** X = S

Chart 2

TABLE II. Taste of Lactones and Lactams Related to 8-Desoxyphyllodulcin



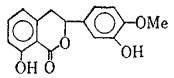
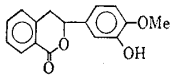
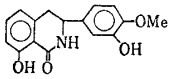
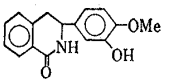
Compd. No.	X	Y	IR cm^{-1} $\nu_{C=O}$	Taste	Compd. No.	X	Y	IR cm^{-1} $\nu_{C=O}$	Taste
6	CH_2	O	1700	sweet(×100)	**10**	CH_2	NH	1658	tasteless
8	O	O	1738	sweet(×100)	**11**	O	NH	1679	tasteless
9	S	O	1707	sweet(×300)	**12**	S	NH	1645	tasteless
—	CH=	O	1712	tasteless	**14**	CH=	NH	1647	tasteless
					13	NH	NH	1638	tasteless

6) Details of the synthesis will be published separately.
7) T.A.K. Smith and H. Stephen, *Tetrahedron*, **1**, 38 (1957).

3. Lactam Derivatives Related to Phyllodulcin

It was found that although 8-desoxyphyllodulcin (× 100) had a sweet taste, the level of its sweetness is weaker than that of phyllodulcin (× 400). This phenomenon may be due to the difference in their structure; presence or absence of a hydroxyl group. Therefore, in order to examine the effect of a hydroxyl group in 8-position on sweet taste, 8-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-3,4-dihydroisocarbostyril (**15**) was synthesized[6] as a lactam derivative related to phyllodulcin. The compound **15** (× 1–10) had a faint sweet taste in contrast with tasteless **10** having no hydroxyl group in 8-position. In the NMR spectra of phyllodulcin and **15**, the signals at 10.82 ppm and 12.71 ppm indicate the proton of 8-hydroxyl group of each compound forms hydrogen bond to adjacent carbonyl group. In the connection, the reason for the apparent effect of 8-hydroxyl group for developing or increasing the level of sweetness may be explained by the hydrogen bond, that is, this hydrogen bond may alter the spatial structure of the lactone or lactam derivatives into desirable form to combine with the site of a sweet receptor.

Table III. Effect of Hydrogen Bond of 8-Hydroxyl Group on Sweet Taste

Compd. No.	Formula	NMR (d_6-DMSO) C_8-OH(δ)	Taste
		10.82	sweet(×400)
6		—	sweet(×100)
15		12.71	sweet(×1—10)
10		—	tasteless

Experimental[8]

***N*-Benzyl-3-hydroxy-4-methoxyaniline (2)**——A solution of 5-nitroguajacol[9] (1.7 g) in EtOH (20 ml) was reduced over 10% Pd-C (0.1 g). After the completion of H_2 absorption the catalyst was filtered off. To the filtrate benzaldehyde (1.0 g) and triethylamine (6 drops) were added and the mixture was refluxed for 1 hr, and the solution was further reduced over 10% Pd-C. After the completion of H_2 absorption, the solvent was removed. The residue was chromatographed over silica gel and elution with CH_2Cl_2 gave **2**, which was distilled under reduced pressure to yield 0.75 g (32.6%) of an oil, bp 180—185° (3 mmHg). NMR (in $CDCl_3$) δ: 3.78 (3H, s, OCH_3), 4.24—4.54 (2H, broad singlet, OH and NH), 6.10 (1H, d.d, J_1=3Hz, J_2=3 Hz, C_6H), 6.32 (1H, d, J=3 Hz, C_2H), 6.72 (1H, d, J=9 Hz, C_5H), 7.36 (5H, singlet with shoulder, aromatic H). Mass Spectrum *m/e*: 229 (M^+). It (×250) has a sweet taste.

3-Hydroxy-4-methoxybenzyl Phenyl Sulfide (4)——A mixture of 3-hydroxy-4-methoxybenzyl alcohol (13.6 g), thiophenol (9.3 g), and dicyclohexylcarbodiimide (DCC) (20.9 g) was sealed in a tube and heated at 100—110° for 24 hr. The reaction mixture was extracted with AcOEt and the solvent was evaporated. The residue was chromatographed over silica gel and eluted with CH_2Cl_2 to give 1.34 g (6.45%) of **4** which

8) All melting points were measured on a micro hot stage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained on a Hitachi Model R-22 spectrometer at 90 MHz employing tetramethylsilane as an internal standard. Mass spectra were measured by a Shimadzu Model LKB-9000 spectrometer. Infrared (IR) spectra were obtained on a Nipponbunko Model DS-301 spectrometer. Ultraviolet (UV) spectra were taken on a Hitachi EPS-2 spectrophotometer.

9) W.A. Jacobs and M. Heidelbelger, *J. Am. Chem. Soc.*, **41**, 1458 (1919).

was recrystallized from cyclohexane, mp 74.5—75.5°. *Anal.* Calcd. for $C_{14}H_{14}O_2S$: C, 68.29; H, 5.69. Found: C, 68.47; H, 5.73. NMR (in $CDCl_3$) δ: 3.75 (3H, s, OCH_3), 3.98 (2H, s, SCH_2), 5.58 (1H, s, OH), 6.12—6.94 (3H, m, aromatic H), 7.20 (5H, singlet with shoulder, aromatic H). It (×150) has a strong sweet taste.

Phenyl 3-Hydroxy-4-methoxythiobenzoate (5)——A solution of 3-hydroxy-4-methoxybenzoic acid (4.0 g), thiophenol (5.1 g), and DCC (5.2 g) in dry dioxane (250 ml) was refluxed for 13 hr. The dioxane was evaporated and the residue was chromatographed over silica gel. Elution with benzene gave 1.7 g (27.4%) of **5**, mp 125—127°. *Anal.* Calcd. for $C_{14}H_{12}O_3S$: C, 64.61; H, 4.61. Found: C, 65.03; H, 4.66. NMR (in $CDCl_3$) δ: 3.90 (3H, s, OCH_3), 5.80 (1H, s, OH), 6.86 (1H, d.d, J_1=6 Hz, J_2=2 Hz, C_6H), 7.30—7.61 (7H, m, aromatic H). Mass Spectrum *m/e*: 260 (M^+). It is tasteless.

3-Hydroxy-4-methoxybenzyl Benzoate (7)——To a solution of 3-hydroxy-4-methoxybenzyl alcohol (8.0 g), dry dimethylformamide (DMF) (10 ml), and dry pyridine (10 ml), benzoyl chloride (10 g) in dry DMF (10 ml) was added dropwise under cooling with ice-water. The mixture was stirred for 8 hr at room temperature. The product was poured into ice and extracted with ether. The ether layer was washed with 10% H_2SO_4 and 10% $KHCO_3$ successively, and after evaporation of the solvent, the residue was chromatographed over alumina. Earlier elution with benzene gave 3-acetoxy-4-methoxybenzyl benzoate (5.6 g, mp 99—102°) and next elution with ether containing 2% acetic acid gave 3.0 g (25.0%) of an oil of **7**. NMR (in $CDCl_3$) δ: 3.78 (3H, s, OCH_3), 5.22 (2H, s, $COOCH_2$), 5.82 (1H, broad singlet OH), 6.78 (1H, d, J=8 Hz, C_5H), 6.90 (1H, d.d, J_1=8 Hz, J_2=2 Hz, C_6H), 7.12 (1H, d, J=2 Hz, C_2H), 7.12—7.58 (3H, m, $C_3'H$, $C_4'H$, and $C_5'H$), 8.05 (2H, d.d, J_1=8 Hz, J_2=2 Hz, $C_2'H$ and $C_6'H$). Mass Spectrum *m/e*: 258 (M^+). It has a bitter taste.

2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzodioxan-4-one (8)——A mixture of salicylic acid (6.8 g) and isovanillin (4.5 g) was suspended in dry benzene (100 ml) in CO_2 atmosphere. The reaction mixture was saturated with dry HCl gas, refluxed for 20 hr, and extracted with AcOEt. The AcOEt layer was washed with 10% $KHCO_3$ and the solvent was evaporated. The residue was chromatographed over silica gel and elution with CH_2Cl_2 gave 100 mg (1.9%) of **8**, mp 143.5—144°. NMR (in $CDCl_3$) δ: 3.86 (3H, s, OCH_3), 5.55 (1H, s, OH), 6.37 (1H, s, C_2H), 6.83 (1H, d, J=8 Hz, $C_5'H$), 6.95—7.27 (4H, m, aromatic H), 7.40—7.63 (1H, m, C_8H), 7.94 (1H, d.d, J_1=7 Hz, J_2=2 Hz, C_5H). Mass Spectrum *m/e*: 272 (M^+). It (×100) has a sweet taste.

2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzothioxan-4-one (9)——A mixture of thiosalycylic acid (2.28 g) and isovanillin (2.28 g) was suspended in dry benzene (50 ml) in CO_2 atmosphere. The reaction mixture was saturated with dry HCl gas and refluxed for 2 hr. The mixture was worked up as described above. A crude product thereby obtained was chromatographed over silica gel and eluted with CH_2Cl_2 to give 0.9 g (22.1%) of **9**, mp 125—126°. *Anal.* Calcd. for $C_{15}H_{12}O_4S$: C, 61.85; H, 4.17. Found: C, 62.17; H, 4.24. NMR (in $CDCl_3$) δ: 3.85 (3H, s, OCH_3), 5.68 (1H, s, OH), 6.42 (1H, s, C_2H), 6.83 (1H, d, J=8 Hz, $C_5'H$), 7.00—7.55 (5H, m, aromatic H), 8.10—8.25 (1H, m, C_5H). Mass Spectrum *m/e*: 288 (M^+). It (×300) has a strong sweet taste.

2-(3-Hydroxy-4-methoxyphenyl)-2,3-dihydro-1,3-benzoxazin-4-one (11)——A mixture of salicylamide (3.8 g), isovanillin (3.9 g) benzenesulfonic acid (2.0 g), and $CHCl_3$ (50 ml) was refluxed in a flask fitted with a modified Soxlet extractor containing anhyd. $CaCl_2$ in the thimble. After refluxing for 17 hr, the reaction mixture was extracted with AcOEt, and the AcOEt layer washed with 10% $KHCO_3$. The solvent was evaporated and the residue was chromatographed over silica gel. Elution with $CHCl_3$ gave 160 mg of **11** which was recrystallized from EtOH, mp 200—202°. NMR (in d_6-DMSO) δ: 3.70 (3H, s, OCH_3), 6.20 (1H, s, C_2H), 6.90—7.70 (6H, m, aromatic H), 7.80 (1H, d.d, J_1=8 Hz, J_2=2 Hz, C_5H), 8.80 (0.4H, s, CONH), 9.30 (0.6H, s, HO–C=N). Mass Spectrum *m/e*: 271 (M^+). It is tasteless.

2-(3-Hydroxy-4-methoxyphenyl)-2,3-dihydro-1,3-benzothiazin-4-one (12)——The following procedure was modified from the method of Bohne[10] for other aromatic aldehydes. A solution of isovanillin (4.0 g) and thiosalicylamide (4.0 g) in abs. EtOH (50 ml) was warmed at 75° in CO_2 atmosphere and a stream of dry HCl was passed through until there was no further increase of appearance of precipitate. After refluxing the mixture for 20 min, the product was filtered off and washed with 50% EtOH. Recrystallization from dioxane gave 3.5 g (46.7%) of **12**, mp 230—232°. *Anal.* Calcd. for $C_{15}H_{13}O_3NS$: C, 62.71; H, 4.52; N; 4.87. Found: C, 62.58; H, 4.65; N, 4.63. NMR (in d_6-DMSO) δ: 3.73 (3H, s, OCH_3), 5.94 (1H, singlet with shoulder, C_2H), 6.50—7.10 (3H, m, aromatic H), 7.10—7.58 (3H, m, aromatic H), 7.98 (1H, d.d, J_1=7 Hz, J_2=2 Hz, C_5H), 8.80 (0.6H, d, J=1 Hz, CONH), 9.06 (0.4H, s, HO–C=N). Mass Spectrum *m/e*: 287 (M^+). It is tasteless.

10) H. Bohne and W. Schmidt, *Arch. Pharm.*, **286**, 330 (1953).