Chem. Pharm. Bull. 26(8)2321—2327(1978)

UDC 547.587.51.04.09 : 541.69.04.09

Chemical Structure and Sweet Taste of Isocoumarins and Related Compounds. X. Syntheses of Sweet 5-Hydroxyflavanones and Related Dihydrochalcones[1]

Masatoshi Yamato, Kuniko Hashigaki, Kumiko Mito, and Takaji Koyama

Faculty of Pharmaceutical Sciences, Okayama University[2]

(Received January 5, 1978)

On the basis of the information from our previous studies on structure–sweetness relationships of phyllodulcin and related 3,4-dihydroisocoumarins, syntheses of sweet flavanones and dihydrochalcones are designed. 5-Hydroxyflavanones, VII, IX, and X, and dihydrochlacone XIII has a sweet taste and the others are tasteless. The structure–sweetness relationships of these compounds are discussed. It is considered that the mode of interaction of 5-hydroxyflavanones and Dihydrochalcone (DHC) lacking glycoside group with a sweet receptor seems to be similar to that of 3,4-dihydroisocoumarins.

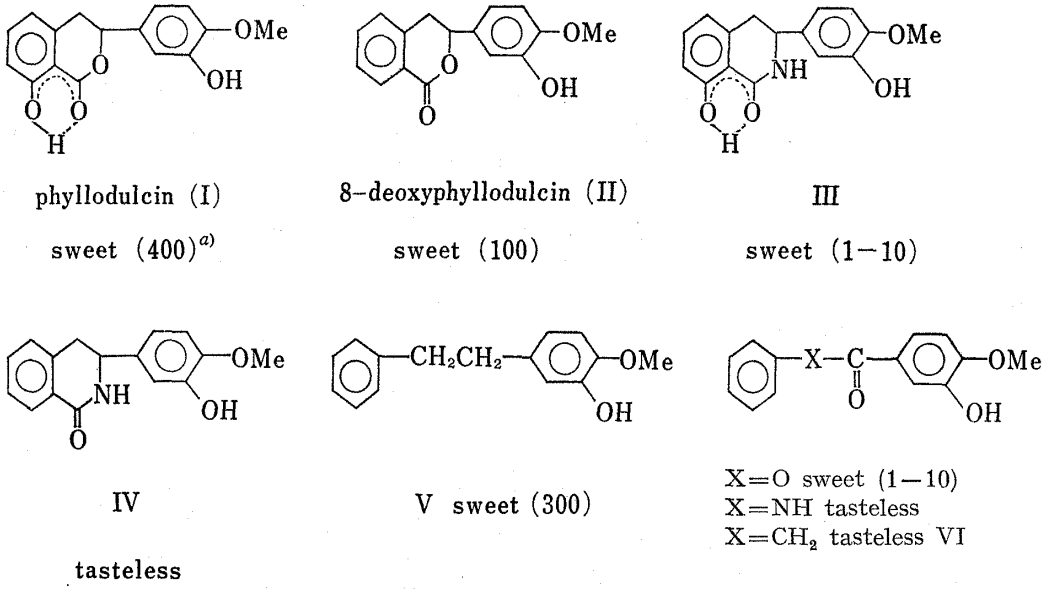
Keywords——structure–sweetness relationship; phyllodulcin; 3,4-dihydroisocoumarins; flavanones; dihydrochalcones

In our previous studies on the structure–sweetness relationships of phyllodulcin and related 3,4-dihydroisocoumarins, many interesting facts have been obtained. Several of them were very peculiar to this series, and were not found in any reports of the studies on other series of sweet compounds. In this work, the information from the structure–sweetness relationship of 3,4-dihydroisocoumarin analogues was applied to design sweet flavanone and dihydrochalcone (DHC) analogues resembling 3,4-dihydroisocoumarin.

Hesperetin is the only sweet flavanone, its sweetness being faint, and the others are bitter or tasteless.[3] The structure–sweetness relationship of flavanones has not yet been studied. While, the DHC glycosides derived from the natural flavanones, prunin, naringin, and neohesperidin, were reported by Horowitz, *et al.* to be potently sweet.[4] Subsequently, the relationship between sweetness and the substituents in the B ring of the DHC 4′-glycosides was studied by Krbechek, *et al.*[5] According to their reports, the glycoside group in the A ring is essential for sweetness and the structural specificity of the glycosyl moiety plays an important role to reveal sweetness. Recently, Crosby, *et al.*[6] and Farkas, *et al.*[7] reported that the compounds in which the glycosyl group of the DHC is replaced by an alkanecarboxylic acid or sulfonic acid group has sweetness.

In our previous studies on 3,4-dihydroisocoumarins and related compounds, following facts were noted, *e.g.*, the level of sweetness of phyllodulcin (I) is higher than that of 8-deoxyphyllodulcin (II), and 3-(3-hydroxy-4-methoxyphenyl)-8-hydroxy-3,4-dihydroisocarbostyril (III) has sweetness, while 3-(3-hydroxy-4-methoxyphenyl)-3,4-dihydroisocarbostyril (IV) is

1) M. Yamato, K. Hashigaki, K. Mito, and T. Koyama, *Chem. Pharm. Bull.* (Tokyo), **25**, 1484 (1977).
2) Location: *Tsushima-naka 1–1–1, Okayama 700, Japan.*
3) R.M. Horowitz and B. Gentli, *J. Agr. Food Chem.*, **17**, 696 (1969).
4) R.M. Horowitz and B. Gentli, U.S. Patent 3087821 (1963).
5) *a*) L. Krbechek, G. Inglett, M. Holik, B. Dowling, R. Wagner, and R. Riter, *J. Agr. Food Chem.*, **16**, 108 (1968); *b*) G. Inglett, L. Krbechek, B. Dowling, and R. Wagner, *J. Food Sci.*, **34**, 101 (1969).
6) G.A. Crosby, G.E. Dubois, and N.M. Weinshenke, U.S. Patent 3974299 (1976); G.A. Crosby and G.E. Dubois, *ibid.*, 3976687 (1976); *C.A.* **85**, 192373 (1976).
7) L. Farkas, M. Nogradi, T. Pfliegel, S. Antus, and A. Gottsegen, *Hung. Teljes*, 10931, *C.A.* **85**, 46201 (1976).



phyllodulcin (I) sweet (400)[a)]

8-deoxyphyllodulcin (II) sweet (100)

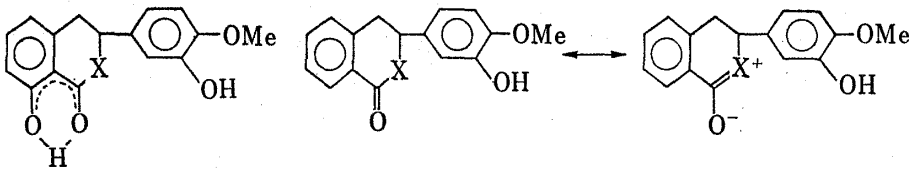
III sweet (1—10)

IV tasteless

V sweet (300)

a) sweetness : 3% sucrose=1

Chart 1



A-form B-form C-form

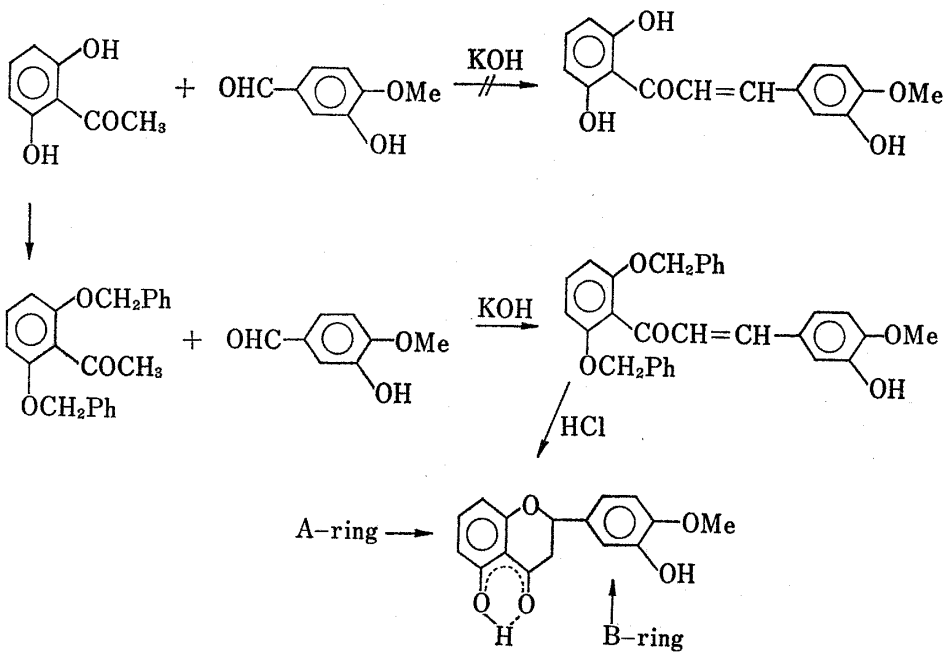
Fig. 1

tasteless (shown in Chart 1).[8] It was concluded from these results that the hydrogen bond of 8-hydroxyl group with 4-carbonyl group of I and III brings about the increase or revealing of sweetness, because I and III having 8-hydroxyl group must exist mainly in A-form, which may be a favorable form for the interaction of I and III with a sweet receptor. While, II and IV lacking 8-hydroxyl group may be an undesirable form for the interaction of II and IV with the sweet receptor owing to the resonance contribution of C-form (shown in Fig. 1). In addition, β-(3-hydroxy-4-methoxyphenyl)ethylbenzene (V) is 300 times sweeter than sucrose and constitutes the essential structure for sweetness of 3,4-dihydroisocoumarins[9] While 3-hydroxy-4-methoxyphenyl benzyl ketone (VI) in which the methylene group of V is replaced by a carbonyl group is tasteless.[10] Namely, it was found that the replacement of the methylene group of V and its bioisosters by a carbonyl group resulted in loss or remarkable decrease of sweetness. For the reason, it was considered that the configuration of these carbonyl analogues may favor a planar configuration owing to the resonance contribution of formula in which a double bond occupies.[10] These fact suggested that the structure

8) M. Yamato, K. Hashigaki, E. Honda, K. Sato, and T. Koyama, *Chem. Pharm. Bull.* (Tokyo), **25**, 695 (1977).
9) M. Yamato, K. Hashigaki, Y. Kuwano, and T. Koyama, *Yakugaku Zasshi*, **92**, 535 (1972).
10) M. Yamato, K. Hashigaki, J. Uenishi, I. Yamakawa, N. Sato, and T. Koyama, *Chem. Pharm. Bull.* (Tokyo), **23**, 3101 (1975).

of flavanones lacking the 5-hydroxyl group seems to resemble to the structure of tasteless VI and would be difficult to combine with a sweet receptor. Moreover, from the fact that the introduction of substituents into the A moiety of V resulted in the remarkable decrease or loss of sweetness with increasing bulkiness of the substituents,[11] it was considered that the substituents at the A ring of natural flavanoness probably cause the loss of sweetness.

On the basis of these information, we presumed that the flavanone having only the 5-hydroxyl group forming hydrogen bond with 4-carbonyl group would be potently sweet. Actually, synthesized 3′,5-dihydroxy-4′-methoxyflavanone (VII) has potent sweetness to be 350 times than sucrose, comparable to that of phyllodulcin. On the other hand, 3′-hydroxy-4′-methoxyflavanone (VIII) lacking the 5-hydroxyl group prepared by the method of Hoerhammer[12] is tasteless, as was expected (shown in Table I). In the course of synthesis of VII, condensation of 2,6-dihydroxyacetophenone and isovanillin did not afford the corresponding chalcone, but when used 2,6-dibenzyloxyacetophenone, the corresponding chalcone was obtained in high yield and, by subsequent hydrolytic cyclyzation, converted to flavanone (VII) (shown in Chart 2).



VII sweet (350)

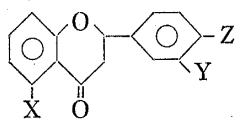
Chart 2

The extended investigation on substituent effects of B ring of the flavanones was undertaken to clarify structure–sweetness relationship of flavanone analogues. Table I shows these results. Replacement of the methoxyl group of VII by an ethoxyl and a *n*-propoxyl groups resulted in a decrease of sweetness with the increasing number of methylenes in the 4′-alkoxyl group. 4′,5-Dihydroxy-3′-methoxyflavanone (XI) in which a hydroxyl and methoxyl groups are introduced into the reversed position in the B ring of VII, is tasteless.

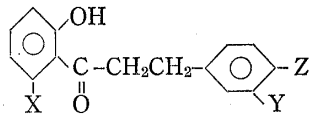
From the results of flavanones, we speculated that the DHC lacking 4′-glycoside group could be modificated to be sweet because of the same reason as in flavanones. Consequently, DHC analogues were synthesized as Table I shows. Among them, 2′,3-dihydroxy-4-methoxydihydrochalcone (XIII) is 100 times sweeter than sucrose, and its structural characteristic

11) M. Yamato, K. Sato, K. Hashigaki, and T. Koyama, *Chem. Pharm. Bull.* (Tokyo), **25**, 706 (1977).
12) L. Hoerhammer, H. Wagner, H. Roesler, M. Keckeisen, and L. Farkas, *Tetrahedron*, **21**, 969 (1965).

Table I. Sweetness of Flavanones and Dihydrochalcones

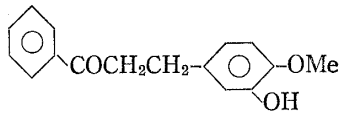


Compd. No.	X	Y	Z	Sweetness[a]
VIII	H	OH	OMe	0
VII	OH	OH	OMe	350
IX	OH	OH	OEt	150
X	OH	OH	O-*n*-Pr	60
XI	OH	OMe	OH	0
XII	OH	H	OH	0



Compd. No.	X	Y	Z	Sweetness[a]
XIII	H	OH	OMe	100
XIV	H	OH	OEt	1—10
XV	H	OH	O-*n*-Pr	0
XVI	OH	OH	OMe	0
XVII	OH	OH	OEt	0
XVIII	OH	OH	O-*n*-Pr	0

a) Sweetness: 3% sucrose=1.

XIX[13]  Acrid

is the existence of the only 2′-hydroxyl group in the A ring. 4-Methoxy-2′,3,6′-trihydroxydihydrochalcone (XVI) is tasteless because of having the two hydroxyl group at 2′- and 6′-positions. Moreover, 3-hydroxy-4-methoxydihydrochalcone (XIX) lacking 2′-hydroxyl group is not sweet.[13] These facts demonstrate that the hydrogen bond of 2′-hydroxyl group with carbonyl group of DHC contributes to reveal sweetness, and that 4′-glycoside moiety is not essential for sweetness of DHC analogues. Replacement of the methoxyl group of XIII by an ethoxyl and propoxyl groups resulted in a decrease of sweetness with the increasing number of methylenes in the 4-alkoxyl group.

Discussion

In our present study the following structure–sweetness relationships were clarified. i) Glycoside moiety is not essential for sweetness of flavanones and DHC. But the 4′-glycoside moiety is necessary for sweetness when the DHC has the 2′- and 6′-hydroxyl groups, which was reported by Krbechek, *et al.*[5] We considered about these fact 6′-hydroxyl group might interact with 4′-glycoside group to bring some effect for revealing of sweetness. ii) The hydroxyl group forming hydrogen bond with the carbonyl group of flavanones and DHC is essential for sweetness. iii) The 3′-hydroxyl and 4′-methoxyl groups are essential for sweetness of flavanones. iv) Exchanging the positions of 3′-hydroxyl and 4′-methoxyl groups of the B ring of flavanones each other resulted in loss of sweetness. These result agrees with those in 3,4-dihydroisocoumarins[14] and DHC 4′-glycosides.[5b] v) The decrease of sweet level attends lengthening the alkyl chain of 4′-alkoxyl group of flavanones, and these results agree with those in 3,4-dihydroisocoumarins and are the reverse of that of DHC 4′-glycosides.[5a] These facts were observed in the DHC lacking glycoside group. vi) The hydroxyl group except 2′-hydroxyl group in the A ring of DHC acts to loss of sweetness.

For the fact that natural and synthetic flavanones hitherto obtained are not sweet but bitter or tasteless, following reasons were considered. i) A hydroxyl and methoxyl groups do not exist in the neighbouring 3′- and 4′-position in their structures. ii) They are lacking

13) M. Sato, *Kagaku Sosetsu*, **14**, 151 (1976).
14) M. Yamato, T. Kitamura, K. Hashigaki, Y. Kuwano, N. Yoshida, and T. Koyama, *Yakugaku Zasshi*, **92**, 367 (1972).

in the hydroxyl group forming hydrogen bond with a carbonyl group. iii) They have some substituents in the benzene ring of chromanone moiety. Because these substituents might project from the plane of the benzene ring and hinder the interaction of the benzene moiety with a sweet receptor.

On the basis of the information obtained from our present study, it was demonstrated that the binding site of DHC 4′-glycosides with a sweet receptor differ from that of DHC lacking 4′-glycoside group, and the latter DHC, flavanones, and 3,4-dihydroisocoumarin analogues must interact with a sweet receptor by a similar mode. In addition, the essential parts of the flavanones and DHC lacking 4′-glycoside group seems to be analogous to V.

Moreover, from the results of the previous molecular modification of V, it was clarified that each of phenyl moiety (A moiety), ethylene moiety (B moiety), and 3-hydroxy-4-methoxyphenyl moiety (C moiety) are playing parts in the appearance of sweet taste respectively.[8),10),11),15)] Namely, the A moiety requires a planarity to reveal a potent sweet taste. The B moiety maintains a definite distance between A- and C-moieties and makes the molecule fit for a steric requirement of a sweet receptor. The 3-hydroxy-4-methoxyphenyl group is suitable for the structure of the C moiety to reveal potent sweet taste. Exchanging the position of 3-hydroxyl and 4-methoxyl groups each other resulted in loss of sweetness.

From those consideration, we propose a gross mode of the interaction of V with a sweet receptor as Fig. 2 shows. The plate of phenyl moiety (A moiety) might stick by van der Waal's force with a flat moiety of a sweet receptor. The 3-hydroxyl and 4-methoxyl groups of the C moiety as the AH and B of the sweet substance,[16a)] holding in a definite distance and spatial structure, might bind by hydrogen bond with the AH and B moiety of a sweet receptor according to the proposal of Schalleberger, *et al.*[16b)]

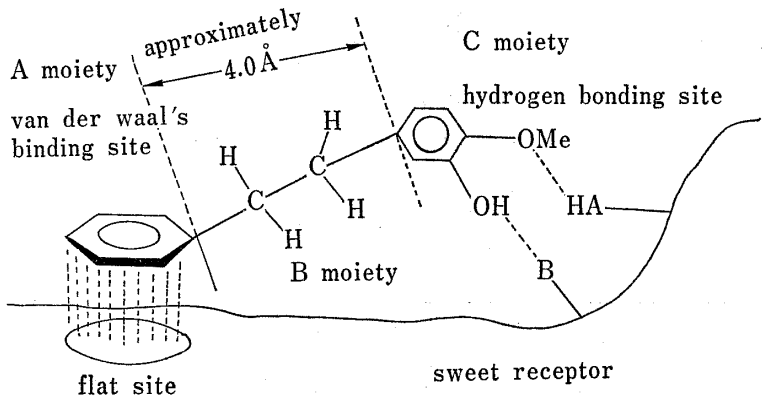


Fig. 2. Gross Mode of Interaction of V with a Sweet Receptor

Experimental[17)]

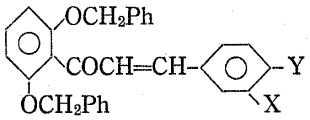
Test of Sweetness——The sweetness of test sample was compared with that of sucrose. An aqueous ethanol solution (H_2O/EtOH=1) of sucrose containing 3% (w/v) was judged to be threshold level. The level of test samples were caluculated from the concentration of the test sample is required to attain the equivalent sweetness to that of 3% sucrose solution.

Synthesis of 2,6-Dibenzyloxychalcones——Benzaldehyde derivatives (0.026 mol) and 2,6-dibenzyloxyacetophenone (0.026 mol) were added to a mixture of 60% KOH aqueous solution (15 ml) and EtOH (30 ml). After stirring at 40° for 6 hr, the reaction mixture was acidified with 10% H_2SO_4 and extracted with AcOEt.

15) M. Yamato, K. Hashigaki, A. Tsukioka, and T. Koyama, *Chem. Pharm. Bull.* (Tokyo), **25**, 700 (1977).
16) *a*) R.S. Schallenberger and T.E. Acree, *Nature* (London), **216**, 480 (1967); *b*) R.S. Schallenberger, T.E. Acree, and C.Y. Lee, *ibid.*, **221**, 555 (1969).
17) All melting points were measured on a hot stage apparatus and are uncorrected.

The AcOEt layer was washed with H_2O and the solvent was removed. Resulting solid was recrystallized to give 2,6-dibenzyloxychalcones. Table II shows the results.

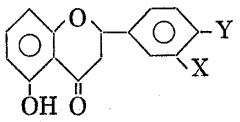
Table II. Synthetic 2,6-Dibenzyloxychalcones



X	Y	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
OH	OMe	150—151	71.0	$C_{30}H_{26}O_5$	77.23	5.62	77.54	5.54
OH	OEt	153—154	95.5	$C_{31}H_{28}O_5$	77.48	5.87	77.65	5.91
OH	O-*n*-Pr	139—141	73.7	$C_{32}H_{30}O_5$	77.71	6.11	77.63	6.25
OMe	OH	52— 54	50.0	$C_{30}H_{26}O_5$	77.23	5.62	77.48	5.59
H	OH	125—127	80.5	$C_{29}H_{24}O_4$	79.79	5.54	80.01	5.49

Synthesis of 5-Hydroxyflavanones——A mixture of the chalcone (0.017 mol), prepared as above, 15% HCl (100 ml), and EtOH (200 ml) was refluxed for 40 hr. The reactant was extracted with AcOEt, and the AcOEt layer was washed with H_2O. Removal of the solvent, the residue was recrystallized to give 5-hydroxyflavanones. Table III shows the results.

Table III. Synthetic 5-Hydroxyflavanones



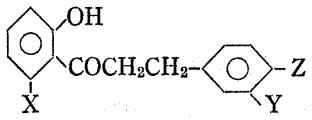
X	Y	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
OH	OMe	136—137	49.2	$C_{16}H_{14}O_5$	67.12	4.93	67.01	5.02
OH	OEt	140—141	35.8	$C_{17}H_{16}O_5$	67.99	5.37	68.18	5.25
OH	O-*n*-Pr	134—135	50.7	$C_{18}H_{18}O_5$	68.78	5.77	68.90	5.65
OMe	OH	163—165	44.2	$C_{16}H_{14}O_5$	67.12	4.93	67.39	4.80
H	OH	206—208	50.5	$C_{15}H_{12}O_4$	70.30	4.72	70.57	4.65

2′,3-Dihydroxy-4-ethoxychalcone (XX)——2-Hydroxyacetophenone (3.9 g) and 4-ethoxy-3-hydroxybenzaldehyde (2.6 g) were added to a mixture of 60% KOH aqueous solution (9 ml) and EtOH (15 ml). After refluxing for 3 hr in N_2 atmosphere, the reaction mixture was worked up as described above for 2′,6′-dibenzyloxychalcones. Resulting solid was recrystallized from MeOH to give 3.3 g (82.9%) of XX, mp 154—155°. *Anal.* Calcd. for $C_{17}H_{16}O_4$: C, 71.82; H, 5.12. Found: C, 72.03; H, 5.09.

2′,3-Dihydroxy-4-*n*-propoxychalcone (XXI)——By treating a mixture of 3-hydroxy-4-*n*-propoxybenzaldehyde (3.3 g) and 2-hydroxyacetophenone (3.9 g) as described above for XX, 3 g (56.0%) of XXI was obtained, mp 139—139.5°. *Anal.* Calcd. for $C_{18}H_{18}O_4$: C, 72.46; H, 6.08. Found: C, 72.61; H, 6.15.

Synthesis of Dihydrochalcones——A solution of the flavanones (VIII—X) or the chalcones (XX and XXI) (0.007 mol), prepared as above, in 8.5% KOH aqueous solution (30 ml) was hydrogenated in an autoclave for 2 hr at an initial pressure of 60 kg/cm³ in the presence of 5% Pd–C (300 mg) at room temperature. The catalyst was removed by filtration, and the filtrate was acidified with 10% H_2SO_4. Resulting precipitate was filtered off and washed with H_2O, and recrystallized to give dihydrochalcones. Table IV shows the results.

TABLE IV. Synthetic Dihydrochalcones



X	Y	Z	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
H	OH	OMe	97— 97.5	34.1	$C_{16}H_{16}O_4$	71.10	5.92	70.97	5.72
H	OH	OEt	81	68.7	$C_{17}H_{18}O_4$	71.31	6.34	71.54	6.41
H	OH	O-*n*-Pr	79	59.5	$C_{18}H_{20}O_4$	71.98	6.71	72.07	6.64
OH	OH	OMe	139—142	54.6	$C_{16}H_{16}O_5$	66.66	5.59	66.85	5.52
OH	OH	OEt	130—131	48.5	$C_{17}H_{18}O_5$	67.54	6.00	67.72	5.89
OH	OH	O-*n*-Pr	129—132	67.2	$C_{18}H_{20}O_5$	68.34	6.37	68.49	6.21