Synthesis and Sweet Taste of Some 2-Phenylbenzodioxanes

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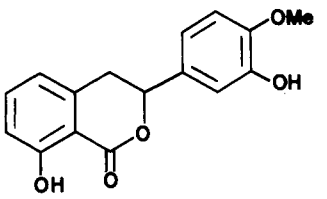
The synthesis of a series of 2-phenyl-1,4-benzodioxanes is reported. One of them, 2-(3-hydroxy-4-methoxyphenyl)-1,4-benzodioxane, is 450 times sweeter than sucrose, with an aftertaste similar to that of Phyllodulcin. Like this latter and other analogues, it satisfies the requirements of the Temussi sweet taste receptor model.

The need of a harmless, intensely sweet, low-caloric, and noncariogenic sweetener is still alive, and research in this field is in continuous progress.

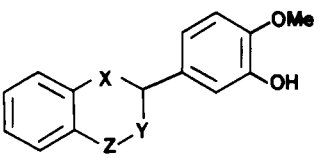
Phyllodulcin (1), a natural dihydroisocoumarin derivative isolated from *Hydrangea thunbergii* Sieboldi, reported to be 400 (Yamato et al., 1977) or 600–800 times sweeter than sucrose (Suzuki et al., 1977) has been the lead for the synthesis of numerous analogues and derivatives, many of them sweet. Studies of the taste–structure relationship for this class of compounds have shown that (a) the 3-hydroxy-4-methoxyphenyl substituent is indispensable for sweet tasting, (b) the heterocyclic B ring must not be planar, and (c) the groups C=O and OH can be both taken away without loss of taste, while products containing only one of them are tasteless or less sweet (Dick, 1981).

The modifications so far operated on the heterocyclic ring B are summarized in Chart I. All the compounds reported are more or less sweet. Particularly interesting is the 1,3-benzodioxane derivative, 3000 times sweeter than sucrose (Dick and Hodge, 1978), which, however, suffers the disadvantage of very low stability in water solution, due to the presence of an acetal moiety. This drawback should not be present in the corresponding 1,4-benzodioxane derivative. As no such compound has been synthesized and tested for sweetness, we undertook this further modification and report here the synthesis of the parent compound **2a** and of a series of derivatives (Chart II).

Chart I

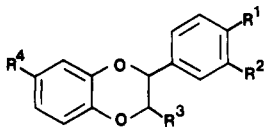


1: Phyllodulcin



X	Y	Z	
CH_2	O	CH_2	isochroman (Yamato et al., 1977)
O	CH_2	CH_2	flavan (Dick, 1981)
O	O	CH_2	1,3-benzodioxan (Dick et al., 1978)

Chart II



CHEMISTRY

The synthesis of compounds **2a–g** was accomplished by the sequence reported in Scheme I. The suitable halo ketones **4** were reacted with a catechol **3** in the presence of a base. The less reactive chloro ketones **4a** required the use of an aprotic dipolar solvent such as DMF (with **3a**) or Me_2SO (with **3b**). In the latter case the use of 1.1 mol of NaH, as recommended by Kessar et al. (1983), did not afford exclusive reaction of the OH para to the electron-withdrawing group, but a 35% yield of a 3:1 mixture of 4- and 3-O-alkylated products. The mixture was used in the following steps and the isomeric ratio measured for the final 1,4-benzodioxane-6-(and 7)-carboxylic acids by capillary GLC of Me_3Si derivatives. Repeated crystallization afforded 96% pure acid **2h**. The choice between the two regioisomeric structures could be made on the basis of the ^{13}C NMR spectrum (Arnone et al., unpublished results).

In the case of **4b** protection of one OH of catechol as benzyl ether was required and the procedure reported (Su et al., 1977) for a similar ketone used. The condensation of bromo ketones **4c–f** with **3a** was accomplished in acetone with K_2CO_3 as a base. Ring–chain tautomerism between the hydroxy ketone and cyclic hemiacetal form (Katritzky et al., 1966), as shown by IR and NMR spectra, was observed for some of the ketones **5**.

Compound	R^1	R^2	R^3	R^4	Taste	log P
2a	OMe	OH	H	H	450 x sucrose	2.8
2b	OMe	OH	H	COOEt	slightly sweet	3.2
2c-cis	OMe	OH	COOEt	H	tasteless	2.8
2c-trans	OMe	OH	COOEt	H	tasteless	3.1
2d	OMe	OH	$CHMe_2$	H	tasteless	3.8
2e	OMe	H	H	H	tasteless	3.8
2f	OMe	H	Me	H	tasteless	4.2
2g	H	H	H	H	tasteless	3.9
2h	OMe	OH	H	COOH	tasteless	1.7
2i	OMe	OH	H	COONa	tasteless	-
2l	OMe	OH	H	CH_2OH	slightly sweet	1.8
2m	OMe	OH	COOH	H	tasteless	1.5
Phyllodulcin						2.6
2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzodioxane						2.7

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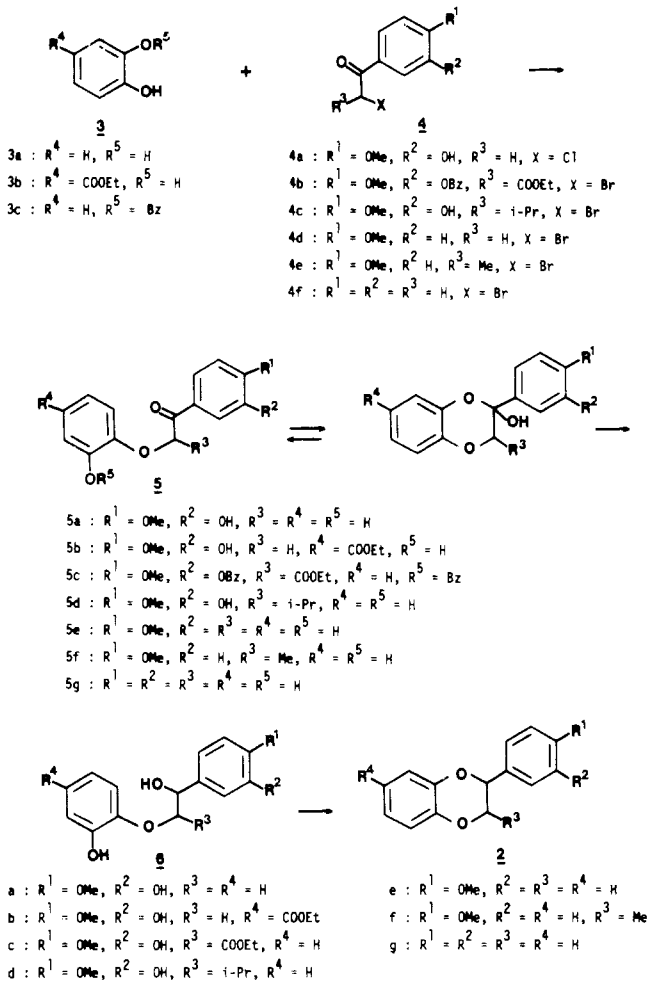
Scheme I



Hydroxy ketones **5** were then reduced by $NaBH_4$ in ethanol to diols **6**. Since compound **5c** is particularly labile, it was reduced in good yield only at room temperature and then it was debenzylated to **6c**; with the inverse sequence only complex mixtures were obtained.

Cyclization of diols **6** to 1,4-benzodioxanes **2** was accomplished by short heating in toluene with the strongly acid ion exchanger Amberlyst 15 as a catalyst. When R^3 was different from H, mixtures of cis and trans compounds were obtained, depending on the substituent on the phenyl ring. A selective procedure by Proietti et al. (1981) gave in our cases too low yields. Cis and trans isomers were tasted separately when possible or in mixture (see the Experimental Section). Compounds **2h** and **2m** were obtained by basic hydrolysis of the corresponding esters **2b** and **2c**.

The alcohol **2l** was obtained by $LiAlH_4$ reduction of the ester **2b** in which the phenolic OH was protected as acetate.

RESULTS AND DISCUSSION

A toxicological classification of 1,4-benzodioxanes as a class cannot be given, as the influence of substituents such as chlorine or aminoalkyl (this latter being present for example in some α-adrenergic antagonists such as piperoxan or idoxan) can change completely the toxicological profile. Some known phenolic 2-phenylbenzodioxanes belong to the neolignan class or natural compounds. Examples include silybin iv LD_{50} = 400 mg/kg in mice (Vogel et al., 1975) and americanin iv LD_{50} = 800 mg/kg in rats (Gorler et al., 1980).

Before tasting, compound **2a** was submitted to preliminary toxicological tests. The acute oral toxicity in rat is $LD_{50} > 5000$ mg kg^{-1}.

Solubility of benzodioxanes **2** in water is low, but stable solutions can be obtained by predissolving them in ethanol and then diluting with deionized water. The solutions of **2a** retained their sweetness after months. Solutions of sucrose in deionized water were prepared as standards. The results of sweet taste measurements of benzodioxanes **2** are shown in Chart II. Compound **2a** is approximately 450 times sweeter than sucrose, with full expression of sweetness requiring 2–3 s. Sweetness lingers a little, and the tongue response to other sweeteners taken immediately afterward is altered for a while. A slight aftertaste of licorice is also present. Compound **2a** has, therefore, a sweetness, flavor, and temporal profile similar to that of Phyllodulcin.

Also in this series the substituents $R^1 = OCH_3$ and $R^2 = OH$ on the phenyl ring have a dramatic effect on the taste, compounds **2e**, **2f**, and **2g** lacking at least one of them are completely tasteless. The two groups are probably indispensable for anchoring the molecule to AH–B system of the receptor (Shallenberger et al., 1967).

The most recent theory of the sweet-taste receptor is that of Temussi (Temussi et al., 1978). He postulated that the receptor is essentially a hemihedral cavity, nearly bidimensional owing to the "Shallenberger barrier", in which only molecules geometrically flat fit well. Dick (1981) suggested that Phyllodulcin fits well in this model in a semiplanar conformation. We think that the benzodioxane derivative **2a** has a conformation quite similar to that of Phyllodulcin and that it can interact in a similar way with the receptor. Benzodioxanes with substituents in position 3 (R^3 = H) can give some information on the requirements of this part of the receptor. All these substitutions (*cis*-**2c**, *trans*-**2c**, **-2d**, **-2m**) induced a strong decrease in sweet taste. Two possible explanations for this fact are that the substituents are too large and they prevent the molecule from entering in the receptor or, owing to their steric hindrance, they compel the phenyl ring in a particular conformation not suitable for a strong interaction with the AH–B system of the receptor. Work is in progress in order to ascertain the preferred conformation for these compounds.

It seems probable that the substituent R^4 has a smaller effect; in fact, the steric requirements of this part of the receptor seem to be less stringent (e.g., sweet dihydrochalcones have large substituents here). Benzodioxanes **2b** ($R^4 = CO_2Et$) and **2l** ($R^4 = CH_2OH$) are less sweet than **2a**, but they are less soluble in water also in the presence of ethanol and their sweetness could not be evaluated exactly. However, the compounds with $R^4 = CO_2H$ (**2h**) and CO_2Na (**2i**) are completely tasteless.

A reviewer has suggested that changes in the hydrophilic lipophilic balance of the molecules could as well explain the strong decrease of sweet taste from **2a** to the substituted compounds. Therefore, we have measured the log *P* of **2a–m**, which are reported in Chart II, together with those of phyllodulcin (**1**) and of 2-(3-hydroxy-4-methoxyphenyl)-1,3-benzodioxane (**7**; Dick and Hodge, 1978). It is interesting to note that the three sweet compound **1**, **2a**, and **7** fall in a short range of log *P*. However no appreciable change in log *P* exists between **2a** and *cis*-**2c**, the latter being tasteless. It may be noticed that the log *P* change induced by the introduction of the same group (CO_2Et) is highly dependent on the stereochemistry (see *cis*-**2c** vs. *trans*-**2c**). It follows that, at least in the benzodioxane series, lipophilicity is not the only important factor for sweetness.

In conclusion, we have shown that also in the 1,4-benzodioxane class it is possible to find sweet compounds, if the basic requirements of the sweet receptor model are satisfied.

EXPERIMENTAL SECTION

Melting points are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$, unless otherwise stated, with Me_4Si as internal standard at 80 MHz on a Bruker WP-80SY spectrometer. Chemical shifts are expressed in ppm. Mass spectra were obtained with a Finnigan 4021 spectrometer equipped with INCOS data system at 70 eV. IR spectra were recorded on a Perkin-Elmer Model 21. Capillary GLC were run on a DANI 3800 gas chromatograph on a SP-2100 30 m × 0.2 mm glass column. Column chromatographies were performed on silica gel Merck 60 (230–400 mesh).

Anydrous DMF, toluene, and dichloromethane were distilled from P_2O_5.

Ketone **4a** (Nodiff et al., 1974) was prepared by a three-step procedure from guaiacol.

The synthesis of benzodioxane **2g** has been already reported (Arnoldi et al., 1983).

Phyllodulcin was purchased from Yuki Gosei Kogyo Co., Ltd., Tokyo. 2-(3-Hydroxy-4-methoxyphenyl)-1,3-benzodioxane was synthesized according to the procedure by Dick and Hodge (1978).

2-Bromo-3-[4-methoxy-3-(benzyloxy)phenyl]-3-oxopropanoic Acid Ethyl Ester (4b). 4-Methoxy-3-(phenylmethoxy)benzoic acid (5 g, 19.4 mmol) was added with 2 drops of pyridine and thionyl chloride (4.5 mL, 62.5 mmol). The solution was refluxed for 1 h and then concentrated at reduced pressure. The crude acid chloride was used in the next reaction without further purification: 4.82 g (90%); mp 77–79°C; IR 1760 cm^{-1}; MS m/z (%) 276 (1), 241 (4), 150 (6), 128 (8), 91 (100). Ethyl acetoacetate (6.84 g, 52.3 mmol) was added dropwise in a slurry of NaH (80% in paraffin oil, 1.5 g, 52.6 mmol) in 6 mL of anhydrous THF; then a solution of the acid chloride (4.82 g, 8.8 mmol) was added in 32 mL of toluene and 4 mL of THF. The solution was refluxed for 10 h, then added with 100 mL of H_2O, and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with water, dried, and concentrated. The residue was refluxed with AcONa (3 g) in 100 mL of ethanol for 4 h. The mixture was filtered, the solid was washed with ethyl acetate, the solvents were concentrated, and the residue was treated with 50 mL of water and extracted with ethyl acetate (3 × 50 mL). 3-[4-Methoxy-3-(benzyloxy)phenyl]-3-oxopropanoic acid ethyl ester was obtained by column chromatography: 4.34 g (75%); mp 62–64 °C (ethanol); lit. mp 61–62 °C (Mangla and Bhakuni, 1980); ^1H NMR δ 1.23 (3 H, t, J=7, CH_3), 3.88 (2 H, s, $COCH_2CO$), 3.93 (3 H, s, OCH_3), 4.21 (2 H, q, J = 7, CH_2), 5.19 (2 H, s, OCH_2Ph), 6.8–7.8 (8 H, arom). A solution of bromine (1.84 g, 11.5 mmol) in 10 mL of chloroform was dropped slowly in a slurry of the β-keto ester (4.05 g, 12.3 mmol) and Na_2CO_3 (2.84 g, 26.8 mmol) in 30 mL of chloroform. The mixture was stirred until disappearance of the brown, then diluted with 30 mL of chloroform, filtered, and evaporated to give the crude bromide as a glassy oil: 5 g, quantitative; ^1H NMR δ 1.24 (3 H, t, J 7, CH_3), 3.93 (3 H, s, OCH_3), 4.24 (2 H, q, J = 7, CH_2), 5.15 (2 H, s, OCH_2Ph), 5.55 (1 H, s, CHBr), 6.88 (1 H, d, J = 9, H-5′), 7.2–7.75 (7 H).

2-Bromo-1-(3-hydroxy-4-methoxyphenyl)-3-methylbutan-1-one (4c). 3-Methylbutanoic acid chloride (14.6 g, 0.12 mol) was dropped at 0 °C into a solution of guaiacol (15 g, 0.12 mol) and pyridine (12.4 g, 0.16 mol) in 80 mL of dichloromethane. The mixture was stirred for 4 h at 20 °C, then washed with two 150-mL portions of 1 M HCl, dried, and concentrated, to give the ester as an oil: 24 g (96%); bp 149 °C; ^1H NMR δ 1.05 (6 H, d, J 6, CH_3), 2.44 (2 H, d, J = 6, CH_2), 1.88–2.55 (1 H, m, H-3), 3.76 (3 H, s, OCH_3), 6.5–7.5 (4 H, arom).

3-Methylbutanoic acid chloride (13.26 g, 0.11 mol) was dropped at 0 °C in a slurry of $AlCl_3$ (19.1 g, 0.143 mol) in 50 mL of CH_2Cl_2; after 0.5 h the guaiacol ester (23 g, 0.11 mol) in 50 mL of dichloromethane was added, and the mixture was stirred at 20 °C for 8 h. The mixture was poured in 300 mL of 3 M HCl and extracted with dichloromethane. After evaporation of the solvent, 1-(4-methoxy-3-hydroxyphenyl)-3-methylbutan-1-one 3-methylbutanoate was obtained as an oil: 29 g (93%); ^1H NMR δ 0.98 (6 H, d, J = 7, 2 CH_3), 1.06 (6 H, d, J = 7, 2 CH_3), 2.0–2.6 (4 H, H-2 and H-2′), 3.93 (3 H, s, OCH_3), 6.7–7.7 (3 H, arom).

This ester (10.22 g, 0.035 mol) was hydrolyzed by refluxing 2 h with 20 mL of 30% NaOH. After treatment with HCl and extraction with ethyl acetate 1-(4-methoxy-3-hydroxyphenyl)-3-methylbutan-1-one was obtained as a solid: 6.53 g (92%); mp 45–48 °C (cyclohexane); ^1H NMR δ 0.96 (6 H, d, J = 7, CH_3), 2.26 (1 H, m, H-3), 2.76 (2 H, d, J = 7, H-2), 3.94 (3 H, s, OCH_3), 6.86 (1 H, d, J = 9), 7.4–7.75 (2 H, arom).

This ketone (0.8 g, 3.85 mmol) in 10 mL of ethyl acetate was added dropwise in a slurry of $CuBr_2$ (1.61 g, 7.2 mmol) in 40 mL of chloroform at reflux. After 2 h the mixture was filtered and the filtrate concentrated to give the 2-bromo ketone **4c**: 1.2 g (79%), oil; ^1H NMR δ 0.99 (3 H, d, J = 7, CH_3), 1.18 (3 H, d, J = 7, CH_3), 2.45 (1 H, m, H-3), 3.90 (3 H, s, OCH_3), 4.90 (1 H, d, H-2), 6.30 (OH), 6.84 (1 H, d, J = 9, H-5′), 7.45–7.75 (2 H, m, arom).

1-(3-Hydroxy-4-methoxyphenyl)-2-(2-hydroxyphenoxy)ethanone (5a). Catechol (1.8 g, 16 mmol) in 10 mL DMF was added dropwise into a slurry of NaH (80% in paraffin oil, 1.44 g, 16 mmol) in 50 mL of DMF. After 15 min, the ketone **4a** (2.8 g, 10 mmol) in 3 mL of DMF was dropped. The solution was stirred at room temperature for 3 h, and then most of the solvent was evaporated in vacuo. The mixture was added with 50 mL of 1 M HCl and extracted with ethyl acetate (3 × 70 mL). The organic solution was washed with water (3 × 70 mL), dried, and concentrated. The residue oil was purified by column chromatography. Compound **5a** was obtained as a white solid: 1.4 g (51%); mp 154 °C (hexane–ethyl acetate, 1:1); MS m/z (%) 274 (21), 166 (9), 151 (100), 137 (9), 123 (9); ^1H NMR (acetone-d_6) δ 3.93 (3 H, s, OCH_3), 6.8–8.1 (7 H, arom), 3.81 (1 H, d, J 11), 4.15 (1 H, d, J = 11, CH_2 of the hemiacetal form), 5.45 (2 H, s, CH_2CO of the hydroxy ketone form).

1-(3-Hydroxy-4-methoxyphenyl)-2-(4-carbethoxy-2-hydroxyphenoxy)ethanone (5b). It was obtained from 3,4-dihydroxybenzoic acid ethyl ester and the ketone **4a** with 1.1 mol of NaH in Me_2SO according to the procedure of Kessar et al. (1983). After workup, the pure ketone **5b** was obtained by column chromatography: 3.58 g (35%); mp 152 °C (toluene); IR 3300, 1700, 1620 cm^{-1}; ^1H NMR δ 1.38 (3 H, t, J = 7, CH_3), 3.99 (3 H, s, OCH_3), 4.34 (2 H, q, J = 7, CH_2), 5.35 (2 H, s, CH_2CO), 5.70 (OH), 6.8–7.7 (7 H, arom + OH); MS m/z (%) 3.46 (8), 301 (2), 166 (7), 151 (100).

3-[4-Methoxy-3-(benzyloxy)phenyl]-2-[2-(benzyloxy)phenoxy]-3-oxopropanoic Acid Ethyl Ester (5c). A mixture of 2-(benzyloxy)phenol (Nelson et al., 1977) (2.88 g, 14.4 mmol), 18-crown-6 (0.29 g, 1.1 mmol), and K_2CO_3 (4 g, 29 mmol) in 60 mL of acetonitrile was stirred for 2 h at 20 °C. The bromide **4b** (5 g, 12.3 mmol) in 70

mL of acetonitrile was added, and the mixture was stirred 20 h, then diluted with 150 mL of ethyl acetate, and washed with water (3 × 100 mL). The organic layer was concentrated, and the ketone **5c** was purified by column chromatography: 5 g (77%); viscous oil; ^{1}H NMR δ 1.17 (3 H, t, J = 7, CH_3), 3.9 (3 H, s, OCH_3), 4.8 (2 H, q, J = 7, CH_2), 5.06 (2 H, s, OCH_2Ph), 5.1 (2 H, s, OCH_2Ph), 5.75 (1 H, s, H-2), 6.6–8.0 (17-H, arom); MS m/z (%) 331 (2), 241 (25), 91 (100).

2-(2-Hydroxyphenoxy)-1-(4-methoxyphenyl)-ethanone (5e). Catechol (0.7 g, 6.5 mmol), the ketone **4d** (1.5 g, 6.5 mmol), and K_2CO_3 (0.9 g, 6.5 mmol) were refluxed in 10 mL of anhydrous acetone. After 3 h, the mixture was diluted with 30 mL of water and extracted with ethyl acetate (3 × 20 mL). The organic layer was dried and concentrated. The ketone **5e** was obtained by column chromatography: 0.8 g (50%); mp 56 °C (toluene); ^{1}H NMR δ 3.85 (3 H, s, OCH_3), 5.30 (2 H, s, CH_2CO), 6.7–7.1 (6 H), 7.9 (2 H, d, H-2′ and H-6′); MS m/z (%) 258 (27), 150 (12), 125 (100), 121 (17), 110 (13), 92 (21), 77 (29).

In the same way were obtained the following:

2-(2-Hydroxyphenoxy)-1-(3-hydroxy-4-methoxyphenyl)-3-methylbutan-1-one (5d): 21%, oil; ^{1}H NMR δ 0.98 (2 H, d, J = 7.0, CH_3), 1.28 (2 H, d, J = 7.0, CH_3), 2.53 (1 H, m, H-3), 3.93 (3 H, s, OCH_3), 5.10 (1 H, d, J = 3.4, H-2), 5.94 (OH), 6.44–7.0 (6 H, arom), 7.2–7.9 (3 H, arom).

2-(2-Hydroxyphenoxy)-1-(4-methoxyphenyl)-propan-1-one (5f): 45%, oil; ^{1}H NMR consistent with a mixture of the hydroxy ketone and the cyclic hemiacetal forms.

1-(3-Hydroxy-4-methoxyphenyl)-2-(2-hydroxyphenoxy)ethanol (6a). Compound **5a** (5.4 g, 19 mmol) and $NaBH_4$ (38 mmol) were refluxed for 2 h in 80 mL of ethanol. The solvent was concentrated under reduced pressure, and the residue was treated with 100 mL of 1 M HCl and extracted with dichloromethane (3 × 100 mL). The oily residue crystallized slowly and was used in the following reaction without further purification: 5 g (96%); mp 56 °C; MS m/z (%) 258 (100, M − 18), 197 (8), 166 (10), 153 (69), 137 (47), 125 (20), 110 (25), 93 (53); ^{1}H NMR (acetone-d_6) δ 2.8 (OH), 3.82 (3 H, s, OCH_3), 3.8–4.2 (2 H, AB of ABX, CH_2), 5.0 (1 H, X of ABX, C*H*OH), 6.7–7.1 (7 H), 7.43 (OH), 7.67 (OH).

In the same way were obtained the following:

2-(4-Carbethoxy-2-hydroxyphenoxy)-1-(3-hydroxy-4-methoxyphenyl)ethanol (6b): 86%; viscous oil; MS m/z (%) 330 (100), 285 (11), 181 (10), 153 (46), 150 (38); H NMR δ 1.33 (3 H, t, J = 7, CH_3), 2.33 (OH), 3.77 (3 H,s,OCH_3), 4.03 (2 H, AB of ABX, CH_2), 4.30 (2 H, q, J = 7, CH_2CH_3), 4.97 (1 H, X of ABX, C*H*OH), 6.5–7.76 (8 H, arom + 2 OH).

1-(3-Hydroxy-4-methoxyphenyl)-2-(2-hydroxyphenoxy)-3-methylbutan-1-ol (6d): 91%; viscous oil; ^{1}H NMR δ 0.88 (3 H, d, J = 7, CH_3), 0.98 (3 H, d, J = 7, CH_3), 1.98 (1 H, m, H-3), 3.78 (3 H, s, OCH_3), 4.78 (1 H, d, J = 5, H-1), 4.8 (OH), 6.3–7.1 (9 H, arom + 2 OH).

2-(2-Hydroxyphenoxy)-1-(4-methoxyphenyl)ethanol (6e): 59%; mp 116 °C (toluene–cyclohexane); ^{1}H NMR δ 1.6 (OH), 3.8 (3 H, s, OCH_3), 4.1 (2 H, AB of ABX, H-2), 5.05 (1 H, X of ABX, H-1), 6.8–7.1 (6 H), 7.35 (2 H, d, H-2′ and H-6′); MS m/z (%) 260 (0.5), 242 (80),137 (100), 134 (52), 121 (47), 110 (27), 94 (11), 91 (12), 77 (18).

1-(4-Methoxyphenyl)-2-(2-hydroxyphenoxy)-propan-1-ol (6f): 75% oil; ^{1}H NMR δ 1.15 (3 H, d, J = 7, CH_3), 3.1 (OH), 3.82 (3 H, s, OCH_3), 3.9–4.6 (1 H, m, H-2), 4.85 (1 H, d, J = 5, H-1), 6.55–7.5 (9 H, arom + 1 OH).

3-[4-Methoxy-3-hydroxyphenyl]-2-(2-hydroxyphenyl)-3-hydroxypropanoic Acid Ethyl Ester (6c). The alcohol **5c** (2.4 g, 4 mmol) was stirred 0.5 h at 20 °C with $NaBH_4$ (4 mmol) in 200 mL of ethanol. After usual workup the benzyl ether of **6c** obtained as a viscous oil, 2 g (95%); MS m/z (%) 333 (2), 286 (11), 243 (7), 135 (13), 121 (27), 91 (100); ^{1}H NMR δ 1.07 (3 H, t, CH_3), 2.82 (OH), 3.84 (3 H, s, OCH_3), 4.01 (2 H, d, CH_2), 4.52 (d, J = 7, H-2 threo isomer), 4.73 (d, J = 5, H-2 erythro isomer), 4.98 (2 H, s, CH_2Ph), 4.99 (1 H, d, J = 5, H-3), 5.06 (2 H, s, CH_2Ph), 6.3–7.7 (17 H, arom). This ether (2 g, 3.8 mmol) was dissolved in 100 mL of ethyl acetate and hydrogenated at 20 °C in the presence of 10% Pd/C (200 mg). The mixture was filtered, evaporated, and chromatographed to give the alcohol **6c**: 0.66 g (50%); mp 124–126 °C (toluene); ^{1}H NMR δ 1.20 (3 H, t, J = 7, CH_3), 2.8 (OH), 3.90 (3 H, s, OCH_3), 4.18 (2 H, q, J = 7, CH_2), 4.68 (1 H, d, J = 5, H-2), 5.14 (1 H, d, J = 5, H-3), 5.61 (OH), 6.6–7.6 (8 H, arom + 1 OH); MS m/z (%) 330 (3), 302 (1), 284 (1), 256 (2), 222 (3), 196 (39), 153 (100), 121 (52), 93 (62). Anal. Calcd for $C_{18}H_{20}O_7$: C, 62.06; H, 5.79. Found: C, 61.98; H, 5.78.

2-(3-Hydroxy-4-methoxyphenyl)-1,4-benzodioxane (2a). Compound **6a** (5 g, 18 mmol) was refluxed for 2 h in 100 mL of toluene with Amberlyst 15 (200 mg). The mixture was filtered, concentrated, and column chromatographed. The benzodioxane **2a** was crystallized twice from cyclohexane: 2.3 g (50%); mp 98–99 °C (cyclohexane); MS m/z (%) 258 (63), 197 (19), 150 (100), 135 (68), 121 (30), 107 (21), 77 (26), 52 (24); ^{1}H NMR δ 3.86 (3 H, s, OCH_3), 3.8–4.4 (2 H, AB of ABX, CH_2), 5.00 (1 H, dd, J = 9 and 2, H-2), 5.68 (OH), 6.8–7.1 (7 H, arom). Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found: C, 69.73; H, 5.09.

In the same way were obtained the following:

3-(3-Hydroxy-4-methoxyphenyl)-1,4-benzodioxane-6-carboxylic Acid Ethyl Ester (2b): 73%; mp 80 °C (toluene); ^{1}H NMR δ 1.28 (3 H, t, J = 6, CH_3), 3.81 (3 H, s, OCH_3), 3.9–4.45 (4 H), 4.93 (1 H, dd, J = 8 and 3, H-3), 5.68 (OH), 6.7–7.7 (arom). Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found: C, 65.52; H, 5.62.

***cis*- and *trans*-3-(3-Hydroxy-4-methoxyphenyl)-1,4-benzodioxane-2-carboxylic Acid Ethyl Ester (*cis*-2c and *trans*-2c).** The oily residue obtained by column chromatography was crystallized from cyclohexane–ethyl acetate. The solid was shown to have the cis configuration by ^{1}H NMR (*cis*-**2c**). The mother liquor was concentrated to a yellowish oil that was shown to have the trans configuration by NMR.

***cis*-2c**: 11%; mp 142–144 °C (cyclohexane–ethyl acetate); ^{1}H NMR δ 1.07 (3 H, t, J = 7, CH_3), 3.88 (3 H, s, OCH_3), 4.07 (2 H, q, J = 7, CH_2), 4.92 (1 H, d, J = 3, H-2), 5.37 (1 H, d, J = 3, H-3), 5.55 (OH), 6.6–7.0 (7 H, arom); MS m/z (%) 330 (51), 284 (19), 256 (39), 241 (33), 222 (81), 197 (28), 177 (74), 150 (65), 137 (84), 133 (39), 121 (100), 105 (32). Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found: C, 65.15; H, 5.43.

***trans*-2c**: 21%; oil; ^{1}H NMR δ 0.96 (3 H, t, J 7, CH_3), 3.72 (3 H, s, OCH_3), 3.97 (2 H, d, J = 7, CH_2), 4.50 (1 H, d, J = 6, H-3), 4.96 (1 H, d, J = 6, H-2), 5.6 (OH), 6.6–7.0 (7 H, arom).

2-(3-Hydroxy-4-methoxyphenyl)-3-(methylethyl)-1,4-benzodioxane (2d): 69%; trans:cis = 86:14 by capillary GLC; viscous oil; MS m/z (%) 300 (69), 285 (6), 257 (11), 245 (19), 229 (23), 197 (54), 192 (49), 177 (48), 163 (55), 148 (29), 137 (80), 116 (100); ^{1}H NMR (acetone-d_6) δ 0.94 (3 H, d, J = 7, CH_3), 1.05 (3 H, d, J = 7, CH_3), 1.35–1.85 (1 H, m, $CHMe_2$), 3.83 (3 H, s, OCH_3), 4.00 (1 H, dd, J =

8 and 3, H-3), 6.8–7.6 (8 H, arom and OH).

2-(4-Methoxyphenyl)-1,4-benzodioxane (2e): 68%; mp 49 °C (cyclohexane); ^{1}H NMR δ 3.80 (3 H, s, OCH_3), 4.01 (1 H, m, J = 11 and 9, H-3), 4.31 (1 H, m, J = 11 and 2, H-3), 5.06 (1 H, m, J = 9 and 2, H-2), 6.94 (2 H, d, J = 9, H-3′ and 5′), 6.70–7.15 (4 H, arom), 7.34 (2 H, d J = 9, H-2′ and 6′). Anal. Calcd for $C_{15}H_{14}O_2$: C, 74.36; H, 5.83. Found: C, 74.27; H, 5.78.

2-(4-Methoxyphenyl)-3-methyl-1,4-benzodioxane (2f): 88%; trans:cis = 71:29 by capillary GLC; by crystallization from hexane the trans isomer obtained 96% pure; mp 88 °C (hexane) (lit. mp 88–90 °C) (Krämer et al., 1968); ^{1}H NMR δ 1.16 (3 H, d, J = 6.3, CH_3), 3.82 (3 H, s, OCH_3), 4.11 (1 H, m, H-3), 4.60 (1 H, d, J = 7.9, H-2), 6.94 (2 H, d, J = 9, H-3′ and 5′), 6.73–7.15 (4 H, arom), 7.31 (2 H, d, J = 8.8, H-2′ and 6′).

3-(3-Hydroxy-4-methoxyphenyl)-1,4-benzodioxane-6-carboxylic Acid (2h). A solution of the ethyl ester **2b** (400 mg, 1.21 mmol) and 6 mL of 10% NaOH in 30 mL of methanol was refluxed for 0.5 h. Methanol was evaporated; the residue was treated with concentrated HCl and extracted with chloroform. The solid residue was crystallized from toluene to give the acid **2h**: 250 mg (68%); mp 175 °C (toluene); ^{1}H NMR δ 3.90 (3 H, s, OCH_3), 3.9–4.5 (2 H, AB of ABX, H-2), 5.05 (1 H, dd, J = 9 and 4, H-3), 6.8–7.8 (7 H, arom + 1 OH); MS m/z (%) 302 (100), 165 (39), 150 (69), 135 (56). Anal. Calcd for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67. Found: C, 63.69; H, 4.77.

3-(3-Hydroxy-4-methoxyphenyl)-1,4-benzodioxane-6-carboxylic Acid Sodium Salt (2i). A solution of the acid **2h** (240 mg, 0.79 mmol) in 15 mL of THF was treated with NaH (0.87 mmol). The salt was collected, dissolved in ethanol, and precipitated with ethyl ether: 240 mg (93%); mp 250 °C; ^{1}H NMR (D_2O) δ 3.86 (3 H, s, OCH_3), 4.0–4.6 (2 H, AB of ABX, CH_2), 5.18 (1 H, dd, J = 8 and 2, H-3), 6.9–7.6 (6 H, arom).

3-(3-Hydroxy-4-methoxyphenyl)-1,4-benzodioxane-6-methanol (2l). The ester **2b** (800 mg, 2.5 mmol) was treated with acetic anhydride (5 mL) and AcONa (400 mg) at reflux for 1 h. The mixture was diluted with 1 M HCl (20 mL) and extracted with ethyl acetate (3 × 20 mL). After evaporation of the solvent the oily acetate (700 mg) was reduced by refluxing for 4 h in anhydrous ethyl ether (20 mL) with $LiAlH_4$ (0.175 g, 4.6 mmol). The mixture was quenched with ethyl acetate (3 mL), hydrolyzed with 1 M HCl, (20 mL) and extracted with ethyl acetate (3 × 20 mL). After column chromatography the alcohol **2l** was obtained as an oil: 150 mg (21% from **2b**); ^{1}H NMR (acetone-d_6) δ 3.30 (2 H, br, 20 H), 3.8–4.5 (2 H, AB of ABX, CH_2), 4.53 (2 H, s, CH_2OH), 5.03 (1 H, dd, J = 9 and 4), 6.8–7.1 (6 H, arom).

***trans*-3-(3-Hydroxy-4-methoxyphenyl)-1,4-benzodioxane-2-carboxylic Acid (2m).** The ester *trans*-**2c** (0.5 g, 1.5 mmol) was stirred at room temperature in 15 mL of 5% NaOH in methanol. After 2 h the solvent was concentrated; the residue was diluted with 1 M HCl and extracted with ethyl acetate. The residue was crystallized from toluene: mp 158–159 °C; MS m/z (%) 303 (31), 284 (38), 256 (18), 241 (27), 213 (24), 194 (78), 179 (36), 133 (38), 121 (57), 91 (100); ^{1}H NMR ($CDCl_3$) δ 3.87 (3 H, s, OCH_3), 4.67 (1 H, d, J = 6, H-2), 5.11 (1 H, d, J = 6, H-3), 6.4–7.5 (8 H, arom + 10 H). Anal. Calcd for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67. Found: C, 63.68; H, 4.67.

Partition Coefficients. The log P values (where P is the octanol–water partition coefficient) were calculated by comparison with those of eight reference compounds whose log P values are known (Nys and Rekker, 1974): salicylic alcohol, 0.73; phenoxyacetic acid, 1.26; 4-nitrophenol, 1.91; 4-chlorophenol, 2.39; 2-naphthol, 2.98; naphthalene, 3.37, biphenyl, 4.04; anthracene, 4.45.

The R_M was calculated as mean of three independent determinations (σ_{max} 6%) on Merck RP-18 (0.25-mm-thick) plates using acetonitrile–water (80:20) as eluent.

The dependence of log P from R_M was calculated by regression analysis on the eight reference compounds as $\log P = 2.86 + 2.50R_M$ (n = 8, r = 0.982). This equation was used to calculate the log P of the benzodioxanes and analogues.

Taste Tests. Critical evaluations by a trained taste panel were not done because the compounds would have required extensive preliminary testing for toxicological properties. Stock solutions in ethanol were prepared for each compound at 10–60 mg mL^{-1}, as solubility permitted. Solutions were then diluted to 20–100 mg L^{-1} with distilled water before tasting by a five-person untrained panel. A 3% sucrose solution in distilled water was used as reference.

2a: a 20 mg L^{-1} solution was clearly sweet; a 65 mg L^{-1} solution was judged isosweet as the 3% sucrose solution (the taste lingers a little and the sensitivity for other taste was altered for a while). The other compounds were tasted in comparison with it. **2b**: a 65 mg L^{-1} solution is slightly sweet; it could not be tasted at higher concentration for solubility reasons. *cis*- and *trans*-**2c**: a 60 mg L^{-1} was not sweet and medicinal like. **2d**: a 65 mg L^{-1} solution was medicinal like. **2e**, **2f**, **2g**: 100 mg L^{-1} solutions were medicinal like. **2h** and **2l**: 65 mg L^{-1} solutions were tasteless. **2i**: a 65 mg L^{-1} solution was slightly sweet, but pleasant.

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Registry No. 1, 21499-23-0; **2a**, 99784-08-4; **2b**, 99783-98-9; *cis*-**2c**, 99783-99-0; *trans*-**2c**, 99784-00-6; *cis*-**2d**, 99784-01-7; *trans*-**2d**, 99784-10-8; **2e**, 99784-02-8; *cis*-**2f**, 99784-03-9; *trans*-**2f**, 99784-12-0; **2g**, 99783-81-0; **2h**, 99784-04-0; **2i**, 99784-05-1; **2l**, 99784-06-2; *trans*-**2m**, 99784-07-3; **3a**, 120-80-9; **3b**, 3943-89-3; **3c**, 6272-38-4; **4a**, 55761-46-1; **4b**, 99783-82-1; **4c**, 99783-83-2; **4d**, 2632-13-5; **4e**, 21086-33-9; **4f**, 70-11-1; **4** (R^1 = OMe, R^2 = OBz, R^3 = CO_2Et, X = H), 77513-51-0; **5a**, 99783-86-5; **5b**, 99783-87-6; **5c**, 99783-88-7; **5d**, 99783-90-1; **5e**, 99783-89-8; **5f**, 99783-91-2; **5g**, 42188-49-8; **6a**, 99783-92-3; **6b**, 99783-93-4; **6c**, 99783-97-8; *threo*-**6c** (benzyl ether), 99784-09-5; *erythro*-**6c** (benzyl ether), 99784-11-9; **6d**, 99783-94-5; **6e**, 99783-95-6; **6f**, 99783-96-7; **7**, 66267-37-6; $Me_2CHCH_2C(O)Cl$, 108-12-3; *o*-*i*-$PrCH_2CO_2C_6H_4OMe$, 68983-11-9; 4-methoxy-3-(phenylmethoxy)benzoic acid, 58452-00-9; 4-methoxy-3-(phenylmethoxy)benzoic acid chloride, 41222-60-0; guaiacol, 90-05-1; 1-(4-methoxy-3-hydroxyphenyl)-3-methylbutan-1-one 3-methylbutanoate, 99783-84-3; 1-(4-methoxy-3-hydroxyphenyl)-3-methylbutan-1-one, 99783-85-4.

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Two Novel Thiophenes Identified from the Reaction between Cysteine and 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone

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From the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) at 160 °C for 0.5 h at pH 2.2 in a closed system, 24 volatile components are identified including two novel compounds, 3-methyl-2-(2-oxopropyl)thiophene and 2-methyl-3-propionylthiophene. These compounds along with 2,4-hexanedione appear to be the major products from the reaction of cysteine and DMHF, based on a comparison of the results obtained from this system with those previously reported from the individual reactants under the similar conditions. The formation mechanism of these thiophenes is proposed.

INTRODUCTION

Investigations of the reactions of α-dicarbonyls with amino acids, especially sulfur-containing amino acids, have provided insight into the products and possible mechanisms operating during the heating of foods (Ho and Hartman, 1982; Hartman and Ho, 1984; Rizzi, 1969). Products formed from Strecker degradation involving sulfur-containing amino acids are believed to be extremely important to the production of food aroma (Vernin, 1982).

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) has been identified in many food sources (Ohloff and Flament, 1979) and used extensively in many flavor areas (Hirvi et al., 1980). The reaction between DMHF and sulfur-containing amino acids would be expected to produce some interesting products chemically and organoleptically and possibly mimic aspects of natural flavor production. In a previous paper (Shu et al., 1985c), the reaction between cystine and DMHF was reported and two novel thiophenones were identified: 2,5-dimethyl-2,4-dihydroxy-3-(2*H*)-thiophenone and 2,5-dimethyl-2-hydroxy-3(2*H*)-thiophenone. In the present study, we report the volatile components generated from the reaction between cysteine and DMHF at 160 °C for 0.5 h in a closed system. Background experiments on the thermal degradation of cysteine (Shu et al., 1985a) and thermal degradation of DMHF (Shu et al., 1985b) were previously reported.

EXPERIMENTAL SECTION

A 500-g mixture was prepared from 0.05 mol of cysteine–HCl–H_2O (Ex. Ajinomoto C., Tokyo, Japan), 0.05 mol of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Ex. International Flavors and Fragrances), and distilled water. The pH of the mixture was measured as 2.2. The mixture was then placed in a 2-L Parr bomb (Parr Instrument Co., Moline, IL) and heated at 160 °C for 0.5 h. The reaction mass was subjected to vacuum steam distillation, extraction, and concentration in that order and the concentrate analyzed by gas chromatography–mass spectrometry (GC–MS) on fused silica columns (OV-1 and Carbowax 20 M) as described previously (Shu et al., 1985a).

In order to isolate and identify the unknown components, 125 mg of the concentrate was separated by column chromatography (10 g of silica gel, 5% H_2O deactivated, methylene chloride–ethyl acetate gradient elution), followed by isolation of the individual components from enriched fractions using gas chromatographic trapping technique (glass capillary columns, 30 m × 0.62 mm, Carbowax 20M and OV-1). The isolates were then characterized by proton nuclear magnetic resonance (NMR, Varian XL-100, $CFCl_3$, relative to internal standard Me_4Si) and infrared (IR, Perkin-Elmer 397) spectra.

RESULTS AND DISCUSSION

The yield of volatiles obtained from the 0.05-mol reaction of cysteine and DMHF was 180 mg. The odor of the mixture of volatiles was described as roasted, bread crust, and meaty. The GC chromatograms of the volatiles are shown in Figures 1 (OV-1 column) and 2 (CWX column). Table I lists the volatile components identified from this sample as compared to the components identified from the thermal degradations of cysteine in water and DMHF in water, respectively. Peak numbers correspond to those in Figures 1 and 2.

Of the components identified, our attention was drawn to peak 17 (OV-1), being the component present in largest concentration. The mass spectrum of peak 17 (Figure 3) suggested that the molecular weight was 154 with one sulfur atom [the (M + 2) peak was 4% of the M peak]. The strong loss of 43 (m/z 111) suggested the presence of acetyl group. The strong absorption of the IR spectrum

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