Free and Glycosidically Bound Volatile Compounds in Fresh Celery (*Apium graveolens* L.)

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The volatiles of celery (*Apium graveolens L.*) were separated into glycosidically bound and free fractions by Amberlite XAD-2 column chromatography combined with direct solvent extraction. The most abundant volatiles were phthalides and psoralens. These are the major volatile compounds found in both the glycosidically bound and free fractions. A total of 34 compounds was identified, including 6 alcohols, 9 terpene and sesquiterpene hydrocarbons, 2 ketones, 7 phthalides, and 5 psoralens. Ten of these have not been previously reported.

INTRODUCTION

The first detailed study on the volatile components of celery stems was conducted by Gold and Wilson (1963a,b), who reported the existence of four unique trace branched-chain alkylidenephthalides, which were claimed to be the characteristic flavor components of celery. During the 1960s, Wilson published a series of papers showing the existence of terpenes and sesquiterpenes (1969a), alcohols (1969b), and carbonyl compounds (1970) in celery essential oils. The compounds that possessed a characteristic celery aroma were identified as β-selinene and phthalides, such as 3*n*-butylhexahydrophthalide, 3*n*-butylphthalide, sedanolide, sedanenolide, 3*n*-butylidenephthalide, and ligustilide.

The literature on celery flavor that concerns the identification and quantitation of phthalides is confusing. Earlier work done by Gold and Wilson (1963a,b) found trace amounts of sedanonic anhydride and several other phthalides in celery. Bjeldanes and Kim (1977) in their work on celery oil isolated the two major phthalide compounds, 3*n*-butylphthalide and 3*n*-butyl-4,5-dihydrophthalide (sedanenolide). They believed sedanenolide to be sedanonic anhydride as reported in the literature. Early identification of sedanenolide and sedanolide, the tetrahydro and dihydro derivatives of 3*n*-butylphthalide, in celery volatiles is also ambiguous. This is probably due to the fact that these two compounds are extremely difficult to resolve on a packed gas chromatographic column. Very recently, Uhlig et al. (1987) reported the separation of these by HPLC. MacLeod et al. (1988) claimed the first separation of these two compounds on a capillary column. Most recently, MacLeod and Ames (1989) compared the volatile components of celery and celeriac and reported that their celery-like odor was associated with each of the 16 phthalides during GC elution.

In this paper, we report the separation of celery flavor compounds into free and glycosidically bound fractions with characterization by GC–MS.

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Table I. Identification of Volatile Components in Fresh Celery

compd	I_k (HP-1)	free fraction, ppm	bound fraction, ppb
3-hexenol	838	0.862	
hexanol	851	0.116	
α-pinene	933	t	
β-pinene	977	0.015	
2-pentylfuran	981	0.035	
phenol	983		0.477
myrcene	984	t	
p-cymene	1016	0.023	
limonene	1027	0.271	
3-carene	1031	t	
γ-terpinene	1154	0.027	
camphor	1131	0.013	
pentylbenzene	1150	0.014	
4-terpineol	1169		0.379
α-terpineol	1182	t	
dihydrocarvone	1187	t	
cis-verbenone	1191	0.023	
trans-carveol	1204	0.032	1.669
β-caryophyllene	1438	0.044	
α-selinene	1484	0.021	
butylphthalide (I)	1629	0.867	5.319
trans-3*n*-butylidene phthalide (II)	1657	0.182	
sedanenolide (III)	1701	20.402	1.426
3-(1′-methylbutylidene)-4,5-dihydrophthalide (tentative)	1702		27.610
cis-ligustilide (IV)	1718	2.179	2.232
sedanolide (V)	1726	4.391	3.958
3*n*-valeryl-4,5-dihydrophthalide (VI)	1767	t	
trans-ligustilide (VII)	1788	t	
psoralen (VIII)	1805	0.273	
scopoletin (IX)	1924	t	5.986
9-methoxypsoralen (X)	1964	0.057	
4-methoxypsoralen (XI)	1975	0.020	
linoleic acid	2004	1.775	
4,9-dimethoxypsoralen (XII)	2031	0.244	

EXPERIMENTAL PROCEDURES

Reagents. The solvents, *n*-pentane, diethyl ether, methanol, and dichloromethane, purchased from Fisher Scientific Co. (Springfield, NJ), were all of HPLC grade and redistilled (except the methanol) prior to use. Amberlite XAD-2 (20–60 mesh) was obtained from Aldrich Chemical Co. (Milwaukee, WI). An almond β-glycosidase was obtained from Sigma Chemical Co. (St. Louis, MO).

0021-8561/90/1438-1937$02.50/0

Table II. Mass Spectral Data for Selected Compounds in Table I

compd	m/z (relative intensity)
butylphthalide	190 (7), 172 (2), 144 (4), 134 (16), 133 (100), 115 (2), 105 (42), 77 (21), 76 (8), 51 (7); M_r 190
trans-3*n*-butylidenephthalide	188 (25), 169 (14), 159 (100), 146 (34), 131 (31), 103 (26), 81 (11), 77 (19), 76 (16), 55 (7); M_r 188
sedanenolide	192 (30), 163 (3), 135 (6), 108 (13), 107 (100), 105 (6), 85 (14), 79 (39), 78 (16), 57 (9), 51 (7); M_r 192
cis-ligustilide	190 (61), 162 (14), 161 (100), 148 (80), 134 (15), 133 (15), 108 (24), 106 (35), 105 (39), 78 (26); M_r 190
sedanolide	194 (11), 137 (42), 109 (62), 108 (100), 81 (38), 80 (56), 77 (18), 79 (82), 53 (13), 41 (20); M_r 194
3*n*-valeryl-4,5-dihydrophthalide	206 (9), 190 (4), 148 (9), 133 (15), 108 (23), 107 (100), 99 (14), 91 (14), 79 (40), 77 (41), 55 (17); M_r 206
trans-ligustilide	190 (59), 162 (14), 161 (100), 148 (83), 134 (15), 133 (23), 106 (44), 105 (60), 91 (16), 78 (35), 77 (34); M_r 190
psoralen	187 (12), 186 (100), 159 (9), 158 (85), 130 (23), 102 (32), 76 (15), 75 (14), 51 (23), 50 (13); M_r 186
scopoletin	193 (15), 192 (100), 177 (65), 165 (36), 149 (72), 121 (32), 81 (14), 79 (23), 69 (39), 51 (19); M_r 192
9-methoxypsoralen	217 (12), 216 (100), 201 (32), 188 (10), 173 (51), 145 (22), 117 (4), 89 (27), 63 (16), 51 (6); M_r 216
4-methoxypsoralen	217 (15), 216 (100), 201 (34), 188 (17), 173 (63), 145 (32), 131 (3), 117 (4), 89 (17), 75 (5); M_r 216
4,9-dimethoxypsoralen	247 (15), 246 (95), 232 (15), 231 (100), 203 (19), 188 (21), 175 (31), 160 (19), 147 (16), 132 (7), 119 (7), 104 (10), 89 (7), 76 (11), 66 (8); M_r 246
3-(1′-methylbutylidene)-4,5-dihydrophthalide (tentative)	204 (31), 176 (13), 175 (100), 162 (36), 147 (33), 134 (7), 119 (13), 103 (5), 92 (10), 91 (20), 77 (5), 65 (7), 63 (9), 55 (8), 51 (4), 39 (6); M_r 204

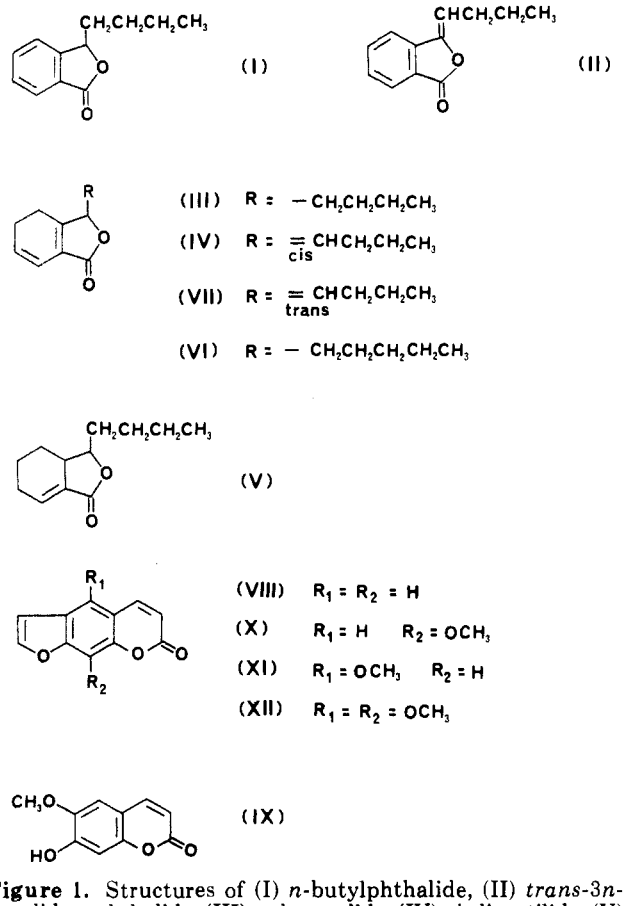

Figure 1. Structures of (I) *n*-butylphthalide, (II) *trans*-3*n*-butylidenephthalide, (III) sedanenolide, (IV) *cis*-ligustilide, (V) sedanolide, (VI) 3*n*-valeryl-4,5-dihydrophthalide, (VII) *trans*-ligustilide, (VIII) psoralen, (IX) scopoletin, (X) 9-methoxypsoralen, (XI) 4-methoxypsoralen, and (XII) 4,9-dimethoxypsoralen.

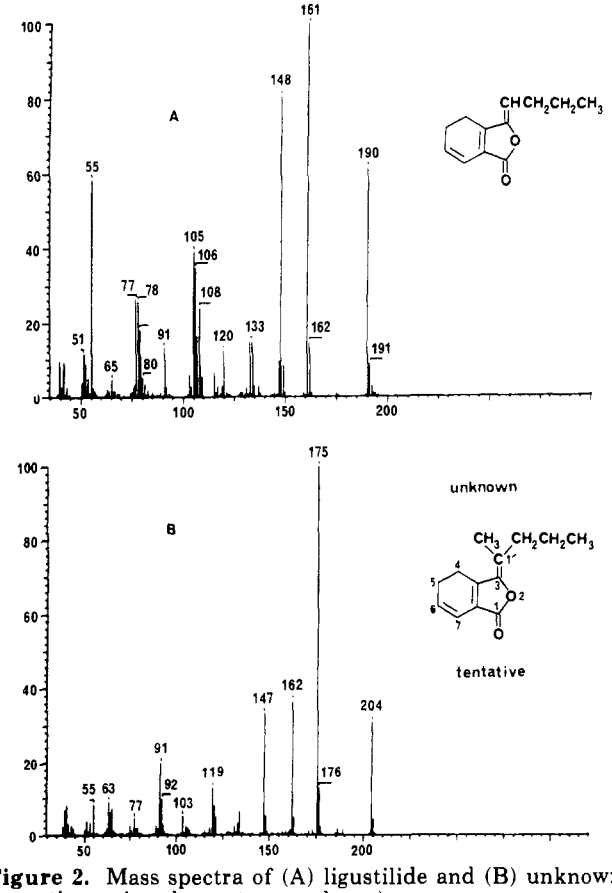

Figure 2. Mass spectra of (A) ligustilide and (B) unknown (tentative assigned structure as shown).

Fresh Pascal celery (*Apium graveolens L.*) was purchased from a local market.

Fractionation of Glycosidically Bound and Free Volatiles. Fresh Pascal celery (4 kg) was washed and cut into half-inch pieces. Afer maceration by a high-shear blender, the fresh juice was passed through a bed of Celite 545 (J. T. Baker Chemical Co., Phillipsburg, NJ), and about 2.7 L of clear juice was obtained. The clear juice was then introduced into a prewashed Amberlite XAD-2 column [1 cm (i.d.) × 50 cm] with a flow rate of 2–2.5 mL/min. The column was rinsed with 100 mL of distilled water to eliminate sugar, acids and other water-soluble substances. A total of 800 mL of pentane/ether 1:1 (v/v) (P/E) was passed through the column at a rate of 2 mL/min to

elute the volatile aroma compounds adsorbed onto the column. The volatile aroma compounds chemically bound as glycosides were eluted with 800 mL of methanol. The methanol was removed by a rotary evaporator and further concentrated to dryness by a stream of nitrogen. The dry material was then dissolved in 100 mL of 0.2 M citrate–phosphate buffer solution (pH 5). The buffer solution was washed three times with 120 mL of P/E 1:1 (v/v) solvent to remove the possible existing traces of free volatiles. The two P/E extracts were combined, dried over anhydrous sodium sulfate, and concentrated to a final volume of 0.5 mL under the stream of nitrogen. This portion is termed the free fraction. The glycosidically bound volatile compounds dissolved in the buffer solution were hydrolyzed by almond β-glucosidase (60 mg, 5.3 units/mg) at 37 °C for 48 h. The liberated aglycons were extracted with dichloromethane (3 × 120 mL), and the extract was dried over anhydrous sodium sulfate and concentrated to a final volume of 0.2 mL with a stream of nitrogen.

Methyl decanoate (1.0 mg/mL) was added as the internal standard to each fraction before concentration.

GC and GC–MS Analysis of Volatile Compounds. A Varian 3400 gas chromatograph equipped with a fused silica capillary column [50 m × 0.32 mm (i.d.); df = 1.05-μm film thickness, HP-1, Hewlett-Packard] and an FID were used to analyze the volatile components in each fraction. The operating conditions were as follows: injection temperature, 270 °C; detector temperature, 300 °C; helium carrier flow rate, 1.0 mL/min; temperature program, 40–230 °C at 2 °C/min and hold at 230 °C for 40 min. A split ratio of 50:1 was used. Quantitative determinations were carried out by using a Varian 4270 integrator. Linear retention indices were calculated against *n*-paraffin standards (C5–C26, Alltech Associates) as references (Majlat et al., 1974).

GC–MS analysis was accomplished by using a Varian 3400 gas chromatograph coupled directly to a Finnigan MAT 8230 high-resolution mass spectrometer. Mass spectra were obtained by electron ionization at 70 eV and recorded on a Finnigan MAT SS 300 data system.

RESULTS AND DISCUSSION

The volatile compounds isolated from the fresh celery are listed in Table I. Their identification, retention indices, and quantitative data are also shown in this table. The quantitation was conducted by computing the area count of each individual compound against that of an internal standard. The internal standard used for this experiment was methyl decanoate, and it is assumed that the response factor for each component equals 1. The assignment of chemical structures was accomplished by comparing the mass spectra with those available either in the National Bureau of Standards (NBS) computer library or from published literature (Heller and Milne, 1980; Ten Noever de Brauw et al., 1983; Swigar and Silverstein, 1981; Toulemonde et al., 1987; Ramaswami et al., 1988). Since the chemical structure assignments for phthalides are quite confusing with regard to the double-bond position and their common names in previously published literature on celery volatile flavors, we are therefore providing our mass spectral data (Table II) as well as the chemical structures (Figure 1). In addition, the mass spectral data and chemical structure assignments for the psoralen-type compounds are listed.

Overall, 34 compounds have been determined in our sample, including some that have not been previously identified. Included are phenol, 3-carene, camphor, *cis*-verbenone, psoralen, scopoletin, 9-methoxypsoralen, 4-methoxypsoralen, linoleic acid, and 4,9-dimethoxypsoralen. For years psoralens have been known as natural chemical components of celery (Windholz et al., 1983) but have not been reported as volatile components. This was probably due to their low volatility. In the present study, the sample preparation was completely carried out at ambient temperatures and the volatiles were isolated by adsorption chromatography combined with direct solvent extraction. This enabled the identification of some new volatile compounds from fresh celery.

(XIII) (XIV)

Figure 3. Structure of (XIII) bavolide and (XIV) dihydrobavolide.

Probably, the most interesting report in celery flavor is the identification of four branched-chain alkyl-substituted phthalides by Gold and Wilson (1963a). Other researchers failed to confirm this finding. In the present study, the most abundant compound in the bound fraction was tentatively identified as branched-chain alkyl-substituted phthalide by comparing the mass spectral data with that of ligustilide (Figure 2). They have a very similar mass spectral fragmentation pattern except that the unknown phthalide contains one methyl group on the ligustilide molecular skeleton. The most probable position of the methyl attachment is on the C1′ as shown in the structure (Figure 2).

As shown in Table I, most phthalides were identified compared with previous studies, and phthalides were the most abundant components for both the free and the glycosidically bound fraction, which accounted for 81.02% and 36.8% of the total isolate, respectively. The quantitation data showed a good agreement with the HPLC analysis results by Uhlig et al. (1987) except for a lower level of butylphthalide. Sedanenolide and sedanolide were found as the major components. It is obvious that phthalides could exist in the form of hydroxy acids, which can be chemically bound to sugars. When the sugar residue is liberated enzymatically or chemically, the aglycons will dehydrate and cyclize to form the related lactones. A large number of γ- and δ-lactones have also been observed in the bound fraction volatiles of pineapple (Wu et al., 1990).

There is significant correlation between the aroma of celery and the individual and total phthalides (Uhlig et al., 1987). Very recently, Mookherjee and Wilson (1990) reported two interesting lactones, bavolide and dihydrobavolide, in tobacco that possess very strong celery-like notes. These two lactones have not, as yet, been identified in celery. The comparison of the structures of these two compounds with those of phthalides (Figure 3) clearly shows a similarity. They both have a five-membered lactone ring with a C-3,4,5-trisubstituted configuration. At position C3, the molecules are substituted with a long alkyl chain which could be saturated or unsaturated at the position between C3 and C1′.

The concentration of related phthalides in the free fraction is 100 times more than in the bound fraction. These results suggest that only a small fraction of the phthalide exists in the glycosidically bound form.

ACKNOWLEDGMENT

New Jersey Agricultural Experiment Station Publication No. 10205-1-90 supported by State Fund and Hatch Regional Project NE-116. We thank Mrs. Joan Shumsky for her secretarial aid.

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Received for review March 12, 1990. Accepted June 11, 1990.

Registry No. I, 6066-49-5; II, 72917-31-8; III, 62006-39-7; IV, 81944-09-4; V, 6415-59-4; VI, 128575-99-5; VII, 81944-08-3; VIII, 66-97-7; IX, 92-61-5; X, 298-81-7; XI, 3380-68-5; XII, 482-27-9; linoleic acid, 60-33-3; 3-hexenol, 544-12-7; 1-hexanol, 111-27-3; α-pinene, 80-56-8; β-pinene, 127-91-3; 2-pentylfuran, 3777-69-3; myrcene, 123-35-3; *p*-cymene, 99-87-6; limonene, 138-86-3; 3-carene, 13466-78-9; γ-terpinene, 99-85-4; camphor, 76-22-2; pentylbenzene, 538-68-1; α-terpineol, 98-55-5; dihydrocarvone, 5948-04-9; *cis*-verbenone, 18309-32-5; *trans*-carveol, 1197-07-5; β-caryophyllene, 87-44-5; α-selinene, 473-13-2.