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RELATIVE RECOVERY AND IDENTIFICATION OF CARBONYL COMPOUNDS FROM CELERY ESSENTIAL OIL

SUMMARY—The carbonyl compounds in celery essential oil obtained from celery leaves and stalks by two essence recovery methods were separated by gas chromatography and identified by chemical and spectroscopic methods. Two epoxides, five ketones, five esters, three acids and three phthalides, 3,n-butylphthalide, sedanolide, and 3,n-butylhexahydrophthalide, were reported as constituents of the essential oil from fresh celery. 3,n-Butylphthalide and sedanolide possess the characteristic odor and flavor of celery. Sixteen of the 18 compounds have not been reported as constituents of the essential oil from fresh celery. Semiquantitative relationships were established for each carbonyl and the carbonyl fractions.

INTRODUCTION

GOLD AND WILSON (1961, 1963a, 1963b) reported the identity of flavoring constituents in celery essential oil that was recovered from celery juice by a vacuum essence recovery technique. The amount of oil available for analysis was extremely small and some identifications were made on the basis of gas chromatographic retention times and functional group analysis. Since some of these identifications were tentative, positive identifications based on chemical and spectroscopic analysis were needed.

Investigations on methods for recovering celery essential oil in high yield provided enough essential oil to conduct an extensive investigation on the chemical composition of celery (Wilson et al., 1967). This paper is the third in a series on the identification and quantitative estimation of the constituents in celery essential oil that was recovered by two different atmospheric steam distillation methods. The first two papers covered the terpene and sesquiterpene hydrocarbons, and alcohols (Wilson 1969a, 1969b). This report covers the isolation, identification and quantitative estimation of the carbonyl compounds in the essential oil recovered from celery stalks and leaves.

EXPERIMENTAL

Isolation of essential oils

The essential oil was recovered by two different methods. In the first, batches of celery puree were steam distilled and the vapors rectified in a packed distillation column (Wilson et al., 1967). In the second, celery puree was pumped into a Turba-Film evaporator and the vapors rectified in a distillation column of different design from the first (Wilson 1969a).

Separation of carbonyl fraction

The essential oils were separated into functional groups by column chromatography on neutral alumina (Fisher A–90, 80–200 mesh) (Wilson 1969a, 1969b). The carbonyls were eluted from the column in three 150 ml fractions with 1:1 v/v hexane-diethyl ether, and the solvent removed in vacuo on a rotary evaporator.

Gas chromatographic analysis

Each carbonyl fraction was separated into individual components by gas-liquid chromatography using the equipment described by Wilson (1969a, 1969b). Stainless steel columns, 15 ft × 0.25 in., packed with 30% Carbowax 20M or 25% Silicone 200 (2,500,000 cstks) on 60–80 mesh Gas Chrom P were used. The helium flow rate was 70 ml/min. Samples were temperature programmed from 100–225°C at 0.5°C/min. Components of the column effluent were collected by trapping in liquid nitrogen cooled glass capillary tubes. Identifications were based on comparisons of GLC retention times with known compounds and by comparison of infrared, NMR and mass spectra with known compounds. The compounds used for comparison purposes were either commercially available or snythesized in the laboratory.

Saponification and synthesis of esters

The esters were saponified by a microsaponification technique, and the alcohol and acid moieties were identified. Saponification was accomplished by placing 25 μl of ethanolic sodium hydroxide (prepared by dissolving one pellet of sodium hydroxide in 0.1–0.2 ml of 50% ethanol) in the glass capillary collection tube with the ester and the mixture was allowed to stand overnight. The reaction mixture was neutralized with 25 μl of concentrated hydrochloric acid, extracted twice with 50 μl of diethyl ether, and then chromatographed. The esters, except for pinocarvyl acetate, were synthesized for comparison purposes by adding acetic anhydride and pyridine 1:2 v/v to the alcohol in a glass capillary collection tube. The mixture was allowed to stand overnight and then chromatographed. Pinocarvyl acetate was synthesized by lead tetraacetate oxidation of

Table 1—Identity and quantitative estimation[a] of major carbonyl constituents in celery essential oil recovered by two different methods.

Column fraction	Constituent	% of Carbonyls: Batch distillation	% of Carbonyls: Continuous distillation[b]
1	t-Limonene oxide	0.50	5.0
	c-Limonene oxide	10.00	5.0
	Dihydrocarvone[c]	5.0	1.5
	Carvone	10.0	5.0
2	α-Ionone	1.0	2.0
	n,Butyl-phenyl ketone	1.0	—
	c,3-Hexenyl-1-acetate	0.25	—
	Pinocarvyl acetate	0.50	5.0
	Dihydrocarvyl acetate	—	1.0
	t-Carvyl acetate	20.0	25.0
	c-Carvyl acetate	2.0	3.0
	c-p-1(7)8-Menthadienyl-2-acetate	2.0	3.0
	Acetic acid	1.0	2.0
	Tiglic acid	0.5	1.0
	Angelic acid	—	0.5
3	3,n-Butylhexahydrophthalide	0.25	0.25
	3,n-Butylphthalide	20.0	20.0
	Sedanolide	5.0	5.0
	Unidentified	21.0	15.75

[a]Quantitative extimations were done by column chromatography and gas-liquid chromatography.
[b]Turba-film evaporator used.
[c]A mixture of cis- and trans-dihydrocarvone.

β-pinene (Gruenewald and Johnson, 1965).

Reduction of phthalides

Identification of 3,n-butylhexahydrophthalide was confirmed by reducing 3,n-butylphthalide and sedanolide to the parent compound. Hydrogenation was done on a Parr pressure-reaction apparatus. 1 ml of 3,n-butylphthalide and sedanolide were each added to 25 ml portions of glacial acetic acid and 50 mg of platinum oxide catalyst. Each mixture was shaken for 24 hr at 80 psi hydrogen pressure.

Pure limonene oxide was processed over neutral alumina, activity 2 (Fisher A–90, 80–200 mesh) to determine if isomerization was occurring (Nigam and Levi, 1968). Limonene oxide was synthesized from limonene and peracetic acid (Newhall, 1959). 5 ml (4.5g) of purified limonene oxide was dissolved in about 25 ml of n-hexane and transferred to a column containing 100g of neutral alumina. The column was eluted with about 150 ml of n-hexane, 200 ml of 1:1 v/v n-hexane-diethyl ether, and then stripped with approximately 150 ml of absolute ethanol. The solvent was removed in vacuo and each fraction was examined by GLC.

Limonene oxide was removed from celery essential oil by high vacuum, low-temperature distillation and the limonene oxide free celery essential oil was subjected to column chromatography. The alcohol fraction was analyzed by GLC (Wilson, 1969b).

Spectrophotometric methods

Infrared spectra were obtained neat on a Perkin-Elmer, Model 137, spectrophotometer. Ultraviolet spectra were obtained on a Cary-14 recoding spectrophotometer. Mass spectra were determined on a Bendix Time-of-Flight, Model 3012, mass spectrometer. Nuclear magnetic resonance spectra (NMR) were taken on a Varian A–60 instrument equipped with a time-averaging computer; carbon tetrachloride was the solvent and tetramethylsilane was the internal standard.

Quantitative comparisons of the two different essential oils were made by estimating the volume of each carbonyl fraction from the neutral alumina column. Quantitative relationships of the individual constituents in each fraction as determined by gas chromatography were established by comparing each constituent with internal standards of identical or similar structure.

RESULTS & DISCUSSION

COLUMN CHROMATOGRAPHY indicated that the carbonyls comprised about 5.0–10.1% (1 and 2 ml respectively) of the two essential oils that were recovered in the continuous and batch distillation units, respectively. Table 1 shows the identity and quantitative estimation of the individual carbonyl compounds. The first fraction eluted from the neutral alumina column consisted of cis- and trans-limonene oxide, cis- and trans-dihydrocarvone, and carvone. Limonene oxide, although it is not a carbonyl, was accounted for because it was isolated with the carbonyls. It is estimated that the first carbonyl fraction accounts for about 25.5% of the total carbonyls in the essential oil obtained by batch distillation, and accounts for about 16.5% of the total carbonyls in the essential oil recovered from the continuous distillation unit.

The second carbonyl fraction of the essential oil recovered by batch distillation contained cis-3-hexenyl-1-acetate, pinocarvyl acetate, cis- and trans-carvyl acetate, cis-1(7)8-p-menthadienyl-2-acetate, α-ionone, n-butyl-phenyl-ketone, and acetic and tiglic acid. These 9 compounds accounted for about 28.5% of the total carbonyls. The second carbonyl fraction of the essential oil recovered by the continuous recovery process contained pinocarvyl acetate, dihydrocarvyl acetate, cis- and trans-carvyl acetate, cis-1(7)8-p-methadienyl-2-acetate, α-ionone; and acetic, tiglic, and angelic acid. These eight compounds accounted for about 42.5% of the total carbonyls.

The third carbonyl fraction consisted primarily of 3 compounds: 3,n-butylphthalide, sedanolide, and 3,n-butylhexahydrophthalide. The ratio of 3,n-butylphthalide to sedanolide was about 4:1 and both of these compounds possessed a strong celery aroma. 3,n-butylhexahydrophthalide was present in trace amounts and its aroma was not as strong as that of 3,n-butylphthalide or sedanolide. The phthalide compounds reported in this study were previously found in celery seed oil and oleo resin (Barton and De Vries, 1963), and have been isolated from other plant sources in the umbelliferae family (Mitsuhashi and Muramatsu, 1964). These compounds have not been reported as constituents of the essential oil from celery stalks and leaves. The third carbonyl fraction accounted for approximately 25.0% of the total carbonyls.

Gold and Wilson (1963) reported the identification of 4 compounds that possessed a characteristic celery aroma and flavor. The previously reported compounds, 3,isobutylidene-3a,4-dihydrophthalide, 3-isovalidene-3a,4-dihydrophthalide, 3-isobutylidenephthalide, and 3-isovalidenephthalide, were not found during the current study. Comparison of chemical and spectral data for the three phthalides reported in the current study with the 4 reported by Gold and Wilson affirms the difference in structure. A possible explanation of the previously reported dihydrophthalides is that in the research reported by Gold and Wilson (1963) large quantities of celery were blanched in flowing steam for 3–4 min and considerable amounts of the steam volatile materials were lost. The quantity of the dihydrophthalides found in the essential oil was extremely small. During the current investigation less celery was used to obtain larger quantities of essential oil. It is conceivable that the dihydrophthalides were present in such small quantities that their presence was not detected in the current study.

The main difference between the oil recovered by batch distillation and that recovered by continuous distillation was in the amount of the more volatile compounds (i.e., the volatile compounds were considered to have gas chromatographic retention times of less than 45 min and the high boiling compounds eluted from the column after 90 min). The oil recovered by continuous distillation had a higher percentage of the more volatile materials. The volatile materials in the oil recovered by continuous distillation accounted for about 10.0% of the unidentified carbonyls. The oil recovered by batch distillation contained very little of the more volatile materials, and the high boiling constituents in this oil accounted for about 20.0% of the unidentified carbonyls.

Column chromatography showed that limonene oxide was readily isomerized by neutral alumina, activity 2. Fractions consisting of 0.1g of cis- and trans-limonene oxide, 0.1g of carbonyls, and 3.5g of alcohols were recovered from the 4.5g of limonene oxide that was placed on the column. The majority of the carbonyl fraction consisted of cis- and trans-dihydrocarvone and carvone. The major constituents in the alcohol fraction were 1-methyl-3-isopropenylcyclopentyl-1-carbonol, cis- and trans-1(7)8-p-menthadiene-2-ol, cis- and trans-carveol, 1,8(10)menthadiene-9-ol, and 8,9-p-menthene-1,2-diol.

Some of the alcohols from limonene oxide isomerization were found in celery essential oil. However, comparison of results obtained from analysis of the alcohols obtained from limonene oxide free celery essential oil and that reported by Wilson (1969b) showed virtually no significant difference.

The organoleptic properties of the carbonyls from the oil recovered by batch distillation and that recovered by continuous distillation were considerably different. The carbonyl fractions from batch distillation possessed a pleasing celery aroma but lacked the notes attributed to the more volatile compounds that were present in the oil recovered by continuous distillation. During the analysis, the odor of each compound was examined as they emerged from the gas chromatograph. All of the carbonyls had a pleasant aroma but there was not any single compound, other than the phthalides, that had an odor characertistic of celery.

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Ms. received 11/10/69; revised 3/6/70; accepted 3/8/70.

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