2-Piperazinemethanethiol Dihydrobromide (Vc).—Reaction of Vb (33.0 g., 96.7 mmoles) with sodium hydrosulfide (0.290 mole) was carried out in the manner described for the preparation of IIId. The reaction mixture was left standing at room temperature for 18 hr. and was then boiled under reflux for 1 hr. in a nitrogen atmosphere. In each subsequent operation the product was protected from air as well as conveniently possible. Removal of the methanol left a white solid residue, from which the product was extracted by two 150-ml. portions of hot acetonitrile. Removal of the acetonitrile gave a semisolid residue that distilled at 140–160° under variable vacuum from a water pump. The slightly yellow crude distillate, which crystallized rapidly, weighed 9.65 g. (75%).

The hygroscopic, air-sensitive free base was dissolved in a solution consisting of 18 ml. of 48% hydrobromic acid in 30 ml. of methanol. When the resulting warm solution was allowed to cool, a copious precipitate deposited; precipitation was completed by addition of acetone. The crude dihydrobromide thus obtained (15.4 g.) was dissolved in 30 ml. of water, the solution was treated with Norit, and the product was precipitated from the filtrate by addition of acetone. This reprecipitation process was repeated and afforded 13.1 g. (47% over-all) of white crystalline powder (dried at 80° *in vacuo* over phosphorus pentoxide); m.p. 220° dec.

Anal. Calcd. for $C_5H_{12}N_2S \cdot 2HBr$: C, 20.42; H, 4.80; N, 9.53; S, 10.90. Found: C, 20.57; H, 4.72; N, 9.29; S, 10.64.

S-2-Piperazinylmethyl Thiosulfuric Acid Hydrobromide (Vd).—A solution of 9.22 g. (27.0 mmoles) of Vb, 3.68 g. (27.0 mmoles) of sodium acetate trihydrate, and 6.70 g. (27.0 mmoles) of sodium thiosulfate pentahydrate in 13.5 ml. of water was heated at 90–95° for 2 hr. After the solution had stood at room temperature for 3 days, a slight amount of crystallization had occurred. The mixture was chilled to 0°, and 150 ml. of cold ethanol was added. The white powder that precipitated was collected and stirred overnight with 50 ml. of methanol. The methanol-insoluble solid was collected (weight 3.00 g.) and stirred 2 hr. in 25 ml. more methanol. The white powder thus obtained was dissolved in 4.0 ml. of hot water, and the solution was filtered with the aid of 2.0 ml. of hot water. The cooled filtrate deposited a small amount of white crystalline precipitate; addition of ethanol completed the precipitation. The white crystalline powder was collected and dried at 78° *in vacuo* over phosphorus pentoxide; yield 2.54 g. (32%); m.p. *ca.* 255° dec.

Anal. Calcd. for $C_5H_{12}N_2O_3S_2 \cdot HBr$: C, 20.48; H, 4.47; S, 21.87. Found: C, 20.52; H, 4.76; S, 22.0.

Acknowledgment.—The authors are indebted to Mr. Carl R. Stringfellow, Jr., for technical assistance, and to members of the Analytical Section of Southern Research Institute, who performed most of the analyses under the direction of Dr. W. J. Barrett. Some of the analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Alkylidene Phthalides and Dihydrophthalides from Celery

Harvey J. Gold and Charles W. Wilson, III

U. S. Fruit and Vegetable Products Laboratory, Southern Utilization
Research and Development Division, Agricultural Research Service,[1] *Winter Haven, Florida*

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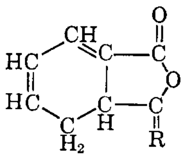
The isolation and characterization of 3-isobutylidene-3a,4-dihydrophthalide (I), 3-isovalidene-3a,4-dihydrophthalide (II), 3-isobutylidene phthalide (III), and 3-isovalidene phthalide (IV) are described. The compounds are believed to be primarily responsible for the flavor and odor of celery.

During the course of investigations on the flavor of the stem and leaf portion of the celery plant, two phthalide and two dihydrophthalide compounds were isolated with the strong characteristic odor of celery. It is proposed that these are 3-isobutylidene-3a,4-dihydrophthalide (I), 3-isovalidene-3a,4-dihydrophthalide (II) and their aromatic analogs.

Following the identification of sedanolide and sedanonic anhydride by Ciamician and Silber[2] as the principal odor constituents of the essential oil of celery seed, Berlingozzi and co-workers[3] undertook a study of the chemistry and odor characteristics of alkyl- and alkylidene phthalides. Working with $\Delta^{2,6}$-dihydrophthalides, Δ^6-tetrahydrophthalides,[4] and hexahydrophthalides, they found that when one of the γ-carbon hydrogens was replaced by an alkyl group, a celery odor was noted. When both were replaced by alkyl groups, the odor was less intense. They note that celery odor was most intense when the γ-carbon hydrogens were replaced by an alkylidene group. Intensity was found to increase as the number of carbons in the group was increased from 1 to 4. The last observation was repeated by Kariyone and Shimizu.[5] The presently reported compounds are naturally occurring materials with structures similar to those of Berlingozzi and co-workers.



I. $R = CH \cdot CH(CH_3)_2$
II. $R = CH \cdot CH_2 \cdot CH \cdot (CH_3)_2$

The compounds were distilled from celery juice under vacuum and the neutral fraction of the distillate extract was chromatographed on silica gel. Final purification was effected by gas chromatography. The compounds gave positive spot tests for the ester group and for unsaturation.[6]

The molecular weight of dihydro compound (I) was found to be 190 using a Mechrolab Osmometer and confirmed by mass spectrometry.[7] The empirical formula was determined as $C_{12}H_{14}O_2$.

The compound was saponified with 10% ethanolic potassium hydroxide. The neutralized solution was partitioned between ether and aqueous sodium bicar-

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[1] Specific mention of particular products or firms does not constitute endorsement by the Department of Agriculture over other products or firms of similar nature.

[2] G. Ciamician and P. Silber, as cited by E. Guenther and D. Althausen, "The Essential Oils," Vol. 2, D. Van Nostrand Co., Inc., New York, N. Y., 1949, pp. 609, 694.

[3] (a) S. Berlingozzi and F. P. Mazzo, *Gazz. chim. ital.*, **56**, 88 (1926); (b) S. Berlingozzi and L. Cione, *ibid.*, **57**, 243 (1927); (c) S. Berlingozzi, *ibid.*, 248 (1927); (d) S. Berlingozzi and G. Lupo, *ibid.*, 255 (1927); (e) S. Berlingozzi, *ibid.*, 264 (1927).

[4] According to current numbering, these would be 5,6-dihydro- and 3a,4,5,6-tetrahydrophthalides.

[5] T. Kariyone and S. Shimizu, *J. Pharm. Soc. Japan*, **73**, 336 (1953).

[6] F. Feigl, "Spot Tests in Organic Analysis," Elsevier Publishing Company, New York, N. Y., 1956, pp. 237, 308.

[7] Petroleum Analytical Research Corporation, 8213 Gulf Freeway, Houston Tex.

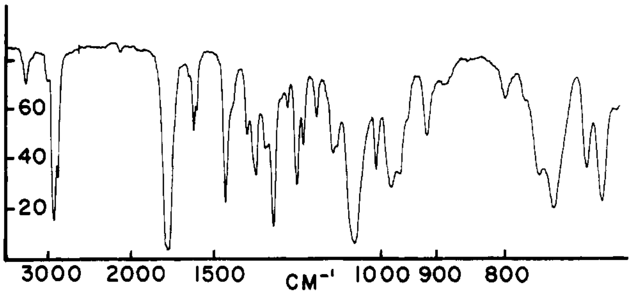

Fig. 1.—Infrared spectrum of 3-isobutylidene-3a,4-dihydrophthalide.

bonate. An acid (characterized by infrared) was obtained from the acidified bicarbonate solution, which reverted to the parent compound during gas chromatography at 175°, establishing the lactone structure.

The principal feature of the mass spectrum was a fragment with mass 134, which coincides with that of the dihydrophthalide nucleus of the proposed structure. The remaining fragment of mass 56 is attributable to the isobutylidene side chain.

In order to confirm the parent structure, the compound was aromatized with sulfur and the resulting material found to be identical with synthesized 3-isobutylidene phthalide by gas chromatographic retention time and by infrared spectrophotometry.

Ozonolysis showed the presence of three ethylenic groups. Reduction of the ozonides gave rise to glyoxal, isobutylaldehyde and an unidentified larger fragment.

The diene residue is shown to be conjugated with the lactone carbonyl by the infrared (Fig. 1) and ultraviolet absorptions. Unsaturation of the γ-carbon of a lactone ring raises the infrared lactone absorption by at least 20 cm.$^{-1}$, unless the α-carbon is also unsaturated.[8] It should be noted that while Bellamy[8] discusses only the situation in which exocyclic γ-unsaturation is combined with endocyclic α-unsaturation, the authors have found that sedanonic anhydride (3-butylidene-3a,4,5,6-tetrahydrophthalide), previously isolated in this laboratory,[9] absorbs at 1775 cm.$^{-1}$. The present compound contains an unsaturated γ-carbon; thus the absorption at 1776 cm.$^{-1}$ shows that the α-carbon must also be unsaturated. Other significant features of the infrared spectrum are absorptions at 690 cm.$^{-1}$, indicating a *cis*-disubstituted ethylene group, and at 750 cm.$^{-1}$ showing a trisubstituted ethylene group.[10]

The ultraviolet absorption at 282 mμ is in agreement with this double bond assignment.[11,12] Woodward's[13,14] rule predicts a wave length of 283 mμ for a diene in conjugation with a carbonyl, α- and δ-carbon substituents and an isolated exocyclic double bond. For other possible double bond assignments, this rule would predict absorptions which differ significantly from any of those observed.

The molecular weight of dihydro compound (II) was found by mass spectrometry to be 204. Its infrared spectrum was virtually identical with that of 3-isobutylidene-3a,4-dihydrophthalide. An additional carbon in the side chain would be expected to leave the infrared spectrum essentially unchanged. Ozonolysis of the compound followed by reduction of the ozonides gave glyoxal and isovaleraldehyde.

A third compound isolated from celery, 3-isobutylidene phthalide, was identified by comparing gas chromatographic retention time and infrared spectrum with the known material prepared as described above.

The 3-isovalidene phthalide was identified by comparison with known material prepared by aromatizing 3-isovalidene-3a,4-dihydrophthalide.

Experimental

Isolation from Celery.—A total of 4130 kg. of celery was steamed to inactivate enzymes. The juice was expressed, vacuum distilled, and the distillate extracted as previously described.[9] Distillation was accomplished in a modified pilot plant essence recovery unit[15] at 15-mm. pressure. The aqueous distillate was extracted with ether in a modified all-glass continuous liquid–liquid extractor.[16] The extract was freed of acidic and phenolic compounds by treatment with aqueous sodium bicarbonate and aqueous sodium hydroxide. Aldehydes and ketones were removed with Girard's T reagent.[17] The remaining fraction was chromatographed on a 1.5 × 20 cm. silica gel column, eluted with hexane, proceeding to hexane–methylene chloride to methylene chloride, to methylene chloride–acetonitrile, and finally to acetonitrile. The dihydrophthalides were eluted with methylene chloride and the phthalides with acetonitrile. Final separation was effected by gas chromatography on a $^1/_4$ in. by 16 ft. column containing 0.25% silicone 200 on glass microbeads at 175° and 10-lb. helium pressure. Total yield was approximately 30 μl. of I and 15 μl. of II and about 5 μl. each of the aromatic compounds. It was established that the conditions used did not cause aromatization of the dihydro compounds.

Isolation from Celery Seed Oil.—Approximately 275 ml. of commercial essential oil of celery seed was freed of acids and phenols, as described above, and the neutral fraction chromatographed on silica gel. The dihydrophthalides were identified in the silica gel effluent by gas chromatography and infrared spectra. The compounds were purified on a $^5/_8$ in. by 16 ft. gas chromatographic column containing 30% Silicone 200 on Gas-Chrom P, operated at 175° and 15-lb. helium pressure. Yield was approximately 200 μl. of I and 70 μl. of II. Only traces of the aromatic compounds were observed.

Ozonolysis.—A sample of 50 μl. (54 mg.) of dihydro compound (I) was dissolved in 1.5 ml. of diethylene glycol, which had first been saturated with ozone, and ozonized for 5.8 min. at room temperature. When known materials were used under these conditions, 85% ozonolysis was obtained. Estimated uptake of ozone for three double bonds: 0.246 mmole; obsd.: 0.219 mmole.

The ozonides were reduced with Raney nickel. Glyoxal and isobutylaldehyde were formed in approximately equimolar quantities and identified by gas chromatographic retention time and infrared spectra of the collected products.

Glyoxal and isovaleraldehyde were identified from a sample of dihydro compound (II) similarly treated.

Aromatization.—Aromatization of the dihydro compounds was carried out according to the procedure of Hershberg and Fieser.[18] A sample of 80 μl. of I was mixed with 1.5 mg. of sulfur in a 4-ml. round bottom flask. The mixture was heated to 230° until the

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(8) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, p. 186.

(9) H. J. Gold and C. W. Wilson, *Proc. Fla. Hort. Soc.*, **74**, 291 (1961).

(10) R. N. Jones, "Infrared Spectra of Organic Compounds: Summary Charts of Principal Group Frequencies," National Research Council Bulletin no. 6, Ottawa, 1959.

(11) R. B. Woodward and G. Singh, *J. Am. Chem. Soc.*, **71**, 758 (1949).

(12) E. A. Braude, in "Determination of Organic Structures by Physical Methods," ed. by E. A. Braude and F. C. Nachod, Academic Press, New York, N. Y. 1955, p. 164.

(13) (a) R. B. Woodward, *J. Am. Chem. Soc.*, **63**, 1123 (1941); (b) R. B. Woodward, *ibid.*, **64**, 76 (1942).

(14) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Company. New York, N. Y., 1949, p. 185.

(15) D. A. Morgan, M. K. Veldhuis, R. K. Eskew, and G. W. M. Phillips, *Food Technol.*, **7**, 382 (1953).

(16) J. J. Kolfenbach, E. R. Kooi, E. I. Fulmer, and L. A. Underkofler, *Ind. Eng. Chem., Anal. Ed.*, **16**, 473 (1944).

(17) C. L. Teitelbaum, *J. Org. Chem.*, **23**, 646 (1958).

(18) E. B. Hershberg and L. F. Fieser in "Organic Syntheses," Coll. Vol. II, A. H. Blatt, ed., John Wiley and Sons, Inc.. New York, N. Y., 1943, p. 423.

sulfur melted and the temperature was gradually raised to 238°. The temperature was held between 238 and and 240° for 30 min. The aromatized compound was taken up in acetone and purified by gas chromatography at 175° using silicone 200 on Gas-Chrom P.

Synthesis of 3-Isobutylidene Phthalide.—The procedure used was that of Weiss.[19] A quantity of 15 g. of phthalic anhydride was mixed with 21 g. of isovaleric acid and 0.5 g. of sodium acetate and heated to 180°. The temperature was allowed to rise to 190° during the next 2 hr. Evolution of carbon dioxide was observed with barium hydroxide for the first 2.5 hr. and heating continued for an additional 2.5 hr. The mixture was freed of unchanged isovaleric acid by washing with aqueous sodium bicarbonate and then taken up in ethyl ether. It was purified by gas chromatography at 175° using silicone 200 on Gas-Chrom P. Yield was about 0.5 ml.; b.p./5 mm.[20]: 83.5° (lit.,[3] 83°). The infrared spectrum of this compound is presented in Fig. 2.

Properties.—All compounds are light yellow oils at room temperature, the aromatic compounds being somewhat darker than the dihydro compounds. They exhibit a greenish fluorescence in agreement with the observations of Berlingozzi and coworkers. In hexane, methylene chloride, or acetonitrile, the dihydro compounds exhibit a blue-green fluorescence. The odors of the various compounds are easily identified as celery in concentrations of 0.1 p.p.m. in water. A number of those who tasted the solutions of the dihydro compounds noted bitterness, and some indicated a burning sensation.

3-Isobutylidene-3a,4-Dihydrophthalide.—*Anal.* Calcd. for $C_{12}H_{14}O_2$: C, 75.8; H, 7.39. Found: C, 75.2; H, 8.0.

Infrared spectra were taken on capillary films between salt plates in a Beckman IR-7 double beam spectrophotometer. For convenience of reproduction, the spectrum shown in Fig. 1 was taken with a Perkin-Elmer Infracord 137 spectrophotometer.

Ultraviolet spectra were taken in a Cary 14 spectrophotometer using 1-cm. fused silica cells. Identical spectra were displayed using acetonitrile and absolute ethanol as the solvent. The spectrophotometer was flushed with dry nitrogen in order to observe the short wave length peaks. Absorptions observed: 282 mμ (log ε, 4.15); 273 mμ (log ε, 4.16); 227 mμ (log ε, 4.61); 197 mμ (log ε, 5.10); b.p./10 mm.,[20] 164°; refractive index $n^{27.5}D$ 1.5092.

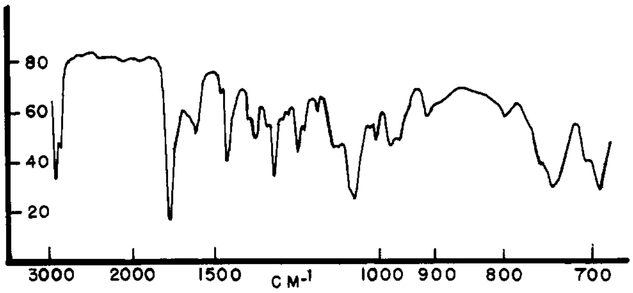

Fig. 2.—Infrared spectrum of 3-isobutylidene phthalide.

Attempts to form derivatives with hydrazine hydrate,[21,22] phenylhydrazine or 2,4-dinitrophenylhydrazine resulted in gums in all cases. Attempts to purify the derivatives by recrystallization resulted in gums of increased viscosity.

3-Isovalidene-3 a,4-Dihydrophthalide.—*Anal.* Calcd. for $C_{13}H_{16}O_2$: C, 76.4; H, 7.85. Found: C, 76.9; H, 8.22.

The infrared spectrum is virtually identical with that shown in Fig. 1 for the isobutylidene compound.

Ultraviolet absorptions: 282 mμ (log ε, 4.12); 273 mμ (log ε, 4.14); 227 mμ (log ε, 4.60); 197 mμ (log ε, 5.08); b.p./10 mm., 175°; refractive index $n^{27.5}D$ 1.5172.

As with the isobutylidene compound, attempts to form crystalline derivatives with the hydrazines were unsuccessful.

3-Isobutylidene Phthalide.—The infrared spectrum is shown in Fig. 2. Ultraviolet absorptions: 280 mμ (log ε, 4.60); 272 mμ (log ε, 4.64); 227 mμ (log ε, 4.89); 200 mμ (log ε, 5.45).

3-Isovalidene Phthalide.—The infrared spectrum is virtually identical with that shown in Fig. 2 for 3-isobutylidene phthalide.

Ultraviolet absorptions: 280 mμ (log ε, 4.56); 272 mμ (log ε, 4.62); 227 mμ (log ε, 4.88); 200 mμ (log ε, 5.32).

Acknowledgment.—The authors are indebted to Dr. G. L. K. Hunter of this laboratory for many suggestions offered during the course of this work, in particular, the suggestion of aromatizing the dihydro compounds, and for his invaluable comments regarding the interpretation of the infrared and ultraviolet spectra. We wish to thank Dr. R. L. Settine of this laboratory for his advice in the application of Woodward's rule.

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Anthocyanins and Related Compounds. I. Structural Transformations of Flavylium Salts in Acidic Solutions

Leonard Jurd

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Albany, California

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Changes in the spectra of a number of flavylium salts, unsubstituted in the 3 position, indicate that in the pH range 1–4 these salts undergo reversible, hydrolytic opening of the pyrylium ring with the formation of the corresponding 2-hydroxychalcones. At pH 4.1, for example, 3′-methoxy-4′,7-dihydroxyflavylium chloride (IV) is predominantly converted (90%) to the chalcone V. Reversible ring opening in weakly acid solutions has been confirmed by the isolation of crystalline chalcones from 8,4′-dimethoxy- and 8-methoxy-2′-hydroxyflavylium chlorides, and by the rearrangement of 5,7,8,4′-tetrahydroxyflavylium iodide in aqueous solution at pH 2.6 to give the isomeric 5,6,7,4′-tetrahydroxyflavylium salt.

The decolorization of anthocyanin pigments in plant extracts is accelerated in the presence of ascorbic acid,[1] sugars,[2] amino acids,[3] and certain enzymes.[4] The products formed and the reactions involved have not yet been determined,[5] although it has been suggested[6] that the rate of anthocyanin destruction in oxygen is pH dependent and directly proportional to the amount of the pigment which exists in the form of the pseudo base. In this connection, therefore, it seemed desirable to verify the structural changes which model flavylium

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