cloud pectin solubilized at pH 7.0 is believed to be due to a conformational change in the cloud protein during its transition from pH 2.5 to pH 7.0, in which the protein remains insoluble (Klavons and Bennett, 1985) and soluble, entrapped pectin is released. This process does not seem to be as efficient as the protease release of soluble pectin, but these two processes appear to be releasing the same type of pectin. When these processes were performed simultaneously (protease treatment at pH 7.0), on the same sample, the soluble pectin released was essentially the same as if the cloud had been treated with pronase alone (Table I).

The pectin that remained insoluble when treated with urea–citrate, 22.1 and 7.4% for samples A and B, respectively, may be classified as "protopectin". Protopectin is regarded as an insoluble precursor to water-soluble pectin and is found in the cell walls (Sinclair, 1984, p 400). Due to the rigorous conditions of juice processing, much of this cell wall material is found in the juice. The exact structure and composition of protopectin are unknown (Sinclair, 1984, p 400), but it has been suggested that protopectin may be composed of two continuous portions: one rich in rhamnose and interposed between blocks of α-1,4-linked D-galacturonic acid and another with a continuous chain of galacturonic acid with various neutral sugars present in side chains (Sinclair, 1984, p 361). The presence of these neutral sugars could account for the water insolubility of protopectin (Sinclair, 1984, p 361). It has been suggested that protopectin plays a role in water retention in fruit tissue and could thus be important in establishing and maintaining fruit consistancy (Sinclair, 1984, p 373).

The remainder of the cloud pectin may then be classified as being inherently insoluble and distinct, that is, not associated with other cloud constituents. Pectin tends to aggregate via polyvalent ions such as calcium, through hydrogen bonding and through other mechanisms (Nelson, 1977; Fishman et al., 1984). Calcium pectate and pectin containing interchain and intrachain hydrogen bonds would be included in this class. The inherently insoluble pectin may be quantitatively represented as that pectin that is solubilized in urea–citrate minus the pectin that is solubilized via protease treatment. Calcium pectate content was determined by its solubilization in sodium oxalate and accounts for this difference.

The degrees of esterification of the total cloud pectin and of that portion of the cloud pectin that may be physically entrapped soluble pectin were not significantly different, and their compositional and/or structural differences remain to be shown.

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Registry No. Pectin, 9000-69-5; citric acid, 77-92-9; urea, 57-13-6; protease, 9001-92-7; sodium oxalate, 62-76-0; protopectin, 9012-27-5; calcium pectate, 12672-40-1.

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Quantitative Analysis of Orange Juice Flavor Volatiles by Direct-Injection Gas Chromatography

Manuel G. Moshonas* and Philip E. Shaw

A distillate was prepared from fresh Valencia or Temple orange juice that possessed all of the fresh orange aroma of the juice. This distillate was analyzed by direct injection into a capillary gas chromatographic column. Of 24 volatile constituents identified, 21 of these were quantitatively measured. Temple orange juice contained lesser quantities of most of these components than did Valencia juice. These values were compared to quantitative estimates of volatile constituents reported earlier in fresh orange juice. The technique can be used to study changes in volatile flavor components due to processing and storage of orange juice products.

The popularity of orange flavor has caused processed orange juice to become the major fruit juice consumed in the United States (Gunter, 1985). Over 200 million boxes of oranges are harvested annually in the United States, making it the largest fruit crop in the country. Extensive research studies have been conducted during the past 30 years in an effort to determine the identities and quantities of volatile components that are important contributors to natural orange flavor and aroma. Such knowledge would be useful for determining flavor changes that occur during processing and storage of orange juice products.

Studies of volatile orange flavor and aroma constituents have historically concentrated on separation and isolation of these compounds from cold-pressed and distilled orange peel oils and from aqueous orange essence, which is the

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early distillate collected during the concentration of fresh orange juice. Although well over 100 compounds have been identified in both peel oil (Shaw, 1977a) and essence (Shaw, 1977b), quantitative analytical measurements on individual components have lagged (Shaw, 1979) because of the difficulties encountered in the isolation of minor components and the losses that occur during separation procedures. Accurate, quantitative data have been reported on less than half of the 109 compounds reported in cold-pressed orange peel oil (Shaw, 1977a, 1979; Vora et al., 1983). Direct injection of the peel oil into the gas chromatograph (GC) made this accurate, quantitative data possible (Shaw and Coleman, 1974). Direct GC injection of aqueous fruit essences, recently reported by Moshonas and Shaw (1984), provided a method for determining accurate, quantitative information on volatile components in those natural flavor fractions. Johnson and Vora (1983) employed this direct-injection method to determine concentration of the major components in aqueous orange essence.

Orange juice volatiles have been analyzed by several workers (Schreier et al., 1977, 1979; Schreier, 1981; Sauri et al., 1980). In all of those studies the analytical procedure involved distillation of juice, extraction with an organic solvent, and concentration of the extract prior to GC analysis. These extraction and concentration steps can cause introduction of artifacts and alterations in the quantitative relationship of volatile constituents. Thus, only limited success has been achieved to date in determining quantitative relationships important to fresh orange juice flavor and aroma.

The present study reports analyses of volatile flavor and aroma constituents of fresh, single-strength orange juice by a simple analytical method that avoids extraction with organic solvents. This method makes it possible, for the first time, to obtain quantitative and qualitative analyses of volatile constituents of single-strength orange juice with a minimum of sample preparation.

EXPERIMENTAL SECTION

Florida Valencia and Temple oranges were purchased at a local market. Samples (four fruit each) were hand-squeezed to obtain 200–250 mL of fresh juice, which was used at once in the following procedure.

Apparatus and Procedure. An aliquot of 100 mL of freshly squeezed orange juice was filtered through two layers of cheesecloth to remove coarse pulp and seeds, and the filtered juice was placed in a 500-mL round-bottomed flask containing a magnetic stirring bar. It was placed in a water bath on a heating plate, which also had magnetic stirring capabilities. The flask was connected to an ice-cooled trap equipped with a chilled water condenser, two liquid nitrogen traps in series, and a vacuum pump as shown schematically in Figure 1. The pressure was maintained at approximately 150 mmHg. The juice was heated to a temperature at or below 60 °C, and 88 mL of aqueous distillate was collected in about 25 min. The remaining thick syrupy residue was devoid of any orange aroma. The vacuum was relaxed, and the first liquid nitrogen trap was slowly heated, allowing the highly volatile material that had condensed on the walls to liquify and combine with the aqueous distillate. None of the volatiles reached the second nitrogen trap. Samples of this aqueous portion were injected directly into a gas chromatograph.

Gas Chromatography. GC data were obtained with a Hewlett-Packard Model 5880A instrument equipped with a flame ionization detector and either a 50-m, wide-bore (0.31–0.32-mm i.d.) fused silica nonpolar capillary column with cross-linked 5% phenylmethyl silicone, film

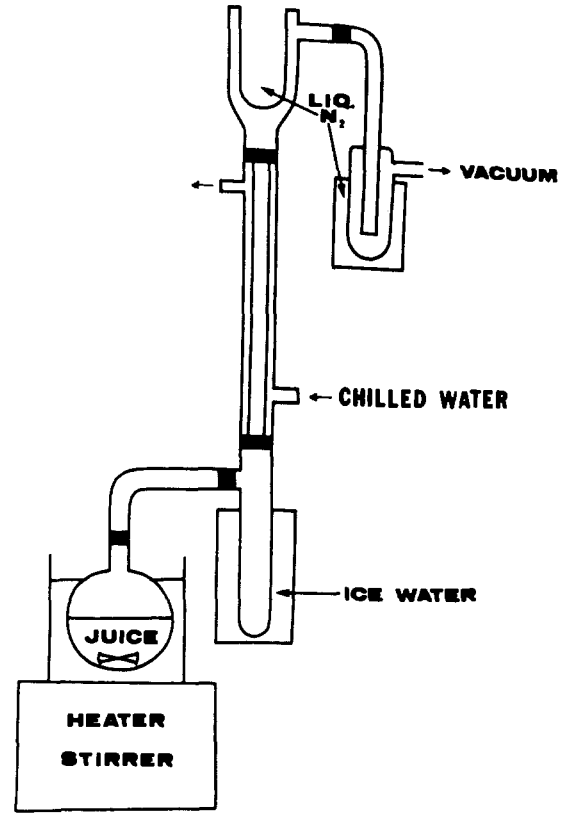

Figure 1. Distillation apparatus.

thickness 1.0 μm (Hewlett-Packard, Avondale, PA), or a 60-m (0.25-mm i.d.) fused silica polar capillary column with DB wax, film thickness 0.25 μm (J&W Scientific, Inc., Rancho Cordova, CA). The capillary inlet system was fitted with a splitless liner that allowed helium to flow down through the liner to the head of the column. There the flow divided, with 1.5 mL/min going through the column, while the rest was vented (Moshonas and Shaw, 1984). This modification minimized discrimination during injection of the sample (Hewlett-Packard, 1980). Injection port and detector temperature was 275 °C. The column temperature was held at 60 °C for 3 min, then programmed to 185 °C at 6 °C/min, and held there for 5 min. Upon completion of the run, the temperature was automatically advanced and kept at 210 °C for 10 min. The threshold was set at 1, peak width at 0.02, and chart speed at 1 cm/min. With the attenuation set at 2↑0, a 10-μL sample of orange juice aqueous distillate was injected into the GC and the oven program started. The large amount of sample injected occasionally blew out the detector flame; the flame was reignited and the run allowed to go to completion, but the data were disregarded. Peaks were identified by sample enrichment with known compounds and by comparison of retention times on polar and nonpolar columns with those for authentic samples.

Response factors for the identified compounds in orange juice distillate were determined by a previously described normalization method (Shaw and Coleman, 1971). The synthetic mixture was prepared in absolute ethanol with standard samples of compounds identified in orange products. The compounds were mixed in the proportions indicated by the GC area percent values determined in Valencia orange juice distillate.

Quantitative Determinations. Standard curves were determined with a series of standard solutions of 1-propanol, ethyl butyrate, and 2-methyl-1-butanol, each at 1, 2, 3, and 4 ppm concentrations by use of relative GC peak heights. These three components were chosen to

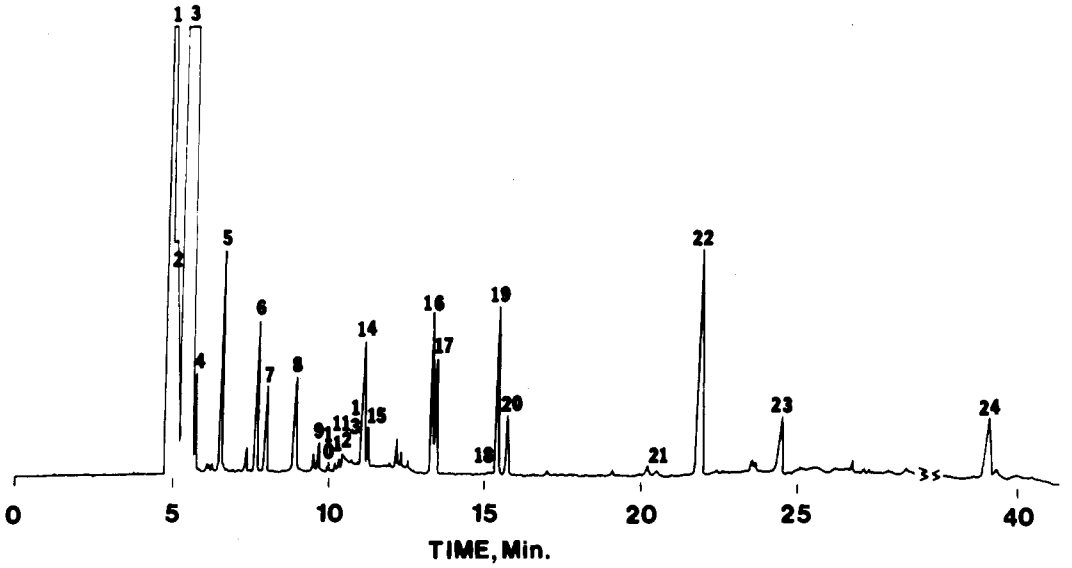

Figure 2. Gas chromatogram of distillate from fresh Valencia orange juice.

represent a large, medium, and small peak in the chromatogram, respectively. After correction with response factors for each of the three compounds, the best linear fit was calculated for each compound ($R^2 > 0.96$ for all three straight lines). The linear equation for the compound whose peak height most closely matched that of the individual component to be quantified was used to calculate each of the values in Table II. These values have been adjusted to reflect 88 ± 1% juice recovery (12% nonvolatiles) in the volatile fraction used to obtain the GC curves.

Aroma Evaluation. Paired comparison tests (Larmond, 1974) were conducted using fresh, single-strength orange juice, and the aqueous distillate was collected from the low-temperature, low-pressure distillation of the same juice. The aroma panel consisted of 12 experienced members, each of whom made two determinations. Panelists were asked to compare the samples and indicate which sample had an aroma more like fresh orange juice.

RESULTS AND DISCUSSION

Quantitative and qualitative analyses of flavor and aroma constituents in fresh, single-strength orange juice were accomplished by an analytical GC method that overcomes major problems caused by very low concentrations of flavor constituents. The presence of soluble solids and large quantities of water has historically prevented direct analysis of citrus and other fruit juices.

An expert flavor panel found no significant differences between the aroma of fresh, single-strength Valencia orange juice and the aroma of the combined distillate collected from the low-temperature, low-pressure distillation employed to separate the aqueous portion of the juice from its soluble solids (Table I). The soluble-solids residue had a weak "haylike" aroma and was totally devoid of orange aroma. These results indicate that no appreciable changes or losses of volatiles had occurred during the separation step. The aroma of the distillate was, however, significantly different from the aroma of canned single-strength orange juice or juice reconstituted from frozen concentrated orange juice (Table I).

The gas chromatograms shown in Figures 2 and 3 were obtained from direct injection of 10-μL samples of aqueous juice distillate from Valencia and Temple oranges. Orange juice constituents identified in this study and the quantity of each determined to be in the juice are listed in Table II.

Table I. Aroma Comparison of Fresh Valencia Orange Juice Distillate with Fresh and Processed Orange Juices

juice sample	judgments pref dist fresh juice[a]	confidence level, %
fresh no. 1	10	NS[b]
fresh no. 2	13	NS
canned single strength	19	99
FCOJ[c]	22	99

[a] Out of 24 total judgments. [b] NS = not significant. [c] Reconstituted commercial frozen orange juice.

Table II. Quantitative and Qualitative Analyses of Fresh Valencia and Temple Orange Juices

peak no.	compd	concn, ppm	
		Valencia	Temple
1	methanol	ND[a]	ND
2	acetaldehyde	ND	ND
3	ethanol	ND	ND
4	acetone	1.5	0.1
5	1-propanol	1.5	0.1
6	ethyl acetate	0.4	0.1
7	2-methylpropanol	0.4	0.1
8	butanol	0.8	0.1
9	1-penten-3-one[b]	0.1	NF[c]
10	2-pentanol[b]	0.1	NF
11	ethyl propionate[b]	0.1	NF
12	methyl butyrate[b]	0.1	NF
13	1,1-diethoxyethane[b]	0.1	NF
14	isoamyl alcohol	1.1	0.4
15	2-methyl-1-butanol	0.3	0.1
16	ethyl butyrate	1.4	1.7
17	hexanol	0.9	NF
18	ethyl 2-methylbutyrate	0.1	NF
19	*cis*-3-hexen-1-ol	0.5	NF
20	*trans*-2-hexenol	0.4	0.1
21	octanal	0.1	NF
22	limonene	1.1	1.8
24	ethyl 3-hydroxyhexanoate	1.0	0.9
24	valencene	0.4	NF

[a] ND = concentration not determined, but positively identified. [b] Tentative identification. [c] NF = not found.

This method makes it possible, for the first time, to obtain detailed quantitative and qualitative data directly from single-strength orange juice. Qualitatively, the volatile constituents identified in the present study had been found earlier in aqueous orange essence (Shaw, 1977b). Five of the compounds listed in Table II are tentatively identified, since they were identified by retention times on only one column (nonpolar). Quantitatively, earlier

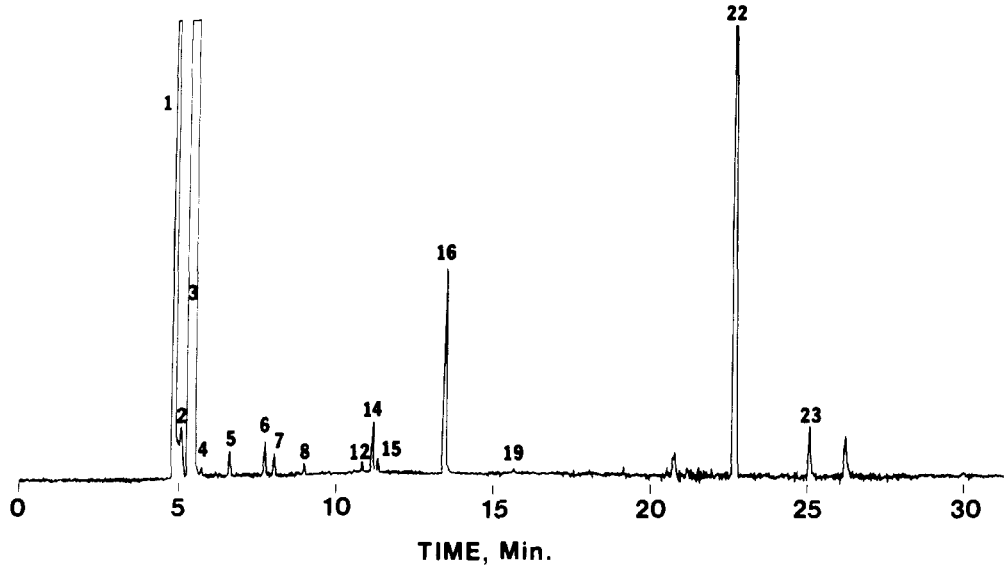

Figure 3. Gas chromatogram of distillate from fresh Temple orange juice.

studies reported estimates of volatile components in fresh orange juice by procedures that involved separation steps that could result in losses of volatiles. Those quantitative studies were reviewed and tabulated by Shaw (1986).

A major difference between this study and earlier ones is that the preponderance of components quantitated in this study were more volatile than limonene, rather than a profile of components with a wide range of volatility. The three major organic components, acetaldehyde, methanol, and ethanol, were not quantitated under these conditions because the peaks representing these components were too large and too poorly resolved for accurate quantitative determinations. Other studies have concentrated on quantitation of these major components in citrus juices (Kirchner and Miller, 1957; Roe and Bruemmer, 1974; Lund et al., 1981).

Of the 16 volatile components positively identified and quantitated in this study, 7 had not been reported in earlier quantitative studies on fresh orange juice (Shaw, 1986). These include acetone, 1-propanol, ethyl acetate, 2-methylpropanol, isoamyl alcohol, ethyl 2-methylbutyrate, and *trans*-2-hexenol. Thus, no comparison with earlier quantitative values was possible for those components. All of the nine remaining components, except butanol and limonene, quantitated earlier in fresh orange juice, were found at higher levels in the present study. The variability in amounts present for all the volatile components could be due to natural variations in fresh fruit samples. The limonene content, in particular, would be expected to vary widely in fresh juice samples, since much of it enters the juice mixture when it is expressed from the peel oil glands during the extraction process.

One important flavor component readily quantitated in Valencia and Temple orange juices by this procedure was ethyl butyrate. This volatile ester is believed to be one of the more important contributors to a desirable orange flavor (Ahmed et al., 1978b; Strobel, 1983, 1984). It has a flavor threshold of 0.13 ppb in water (Ahmed et al., 1978a) and 0.20 ppm in orange juice (Lund, 1985). Thus, this compound is present in these samples of Valencia orange juice at 7 times its flavor threshold in juice and in Temple orange juice at more than 8 times its flavor threshold.

The difference between the gas chromatographic profiles for volatile components of fresh Valencia and Temple orange juices was pronounced (Figures 2 and 3). Valencia orange juice showed a more balanced and stronger profile of volatile components than did Temple orange juice. In the Temple orange sample (Figure 3) the quantity of ethyl butyrate present was disproportionately larger, when compared to most other volatile components present (Table II). These gas chromatographic profiles were reproducible for fresh juice samples, since four samples from the same lot of fresh Valencia orange juice gave virtually identical GC profiles, as did two samples from the same lot of Temple orange juice.

This technique for determining volatile components should provide more accurate quantitative information on many important flavor components in orange juice than has been reported previously. The minimal sample manipulation required prior to injection to the sample into a gas chromatograph should minimize losses or changes in volatile components. Without the distillation step to remove all the volatiles from the sugars and other nonvolatile components (that is, injection of the whole, filtered juice), we obtained an entirely different gas chromatographic pattern. This GC pattern seems largely due to decomposition products from the sugars and is not distinctive for each type of citrus juice. Further work using this simple distillation technique is in progress to monitor changes in composition of orange juice during processing and storage.

Registry No. Methanol, 67-56-1; acetaldehyde, 75-07-0; ethanol, 64-17-5; acetone, 67-64-1; 1-propanol, 71-23-8; ethyl acetate, 141-78-6; 2-methylpropanol, 78-83-1; butanol, 71-36-3; 1-penten-3-one, 1629-58-9; 2-pentanol, 6032-29-7; ethyl propionate, 105-37-3; methyl butyrate, 623-42-7; 1,1-diethoxyethane, 105-57-7; isoamyl alcohol, 123-51-3; 2-methyl-1-butanol, 137-32-6; ethyl butyrate, 105-54-4; hexanol, 111-27-3; ethyl 2-methylbutyrate, 7452-79-1; *cis*-3-hexen-1-ol, 928-96-1; *trans*-2-hexenol, 928-95-0; octanal, 124-13-0; limonene, 138-86-3; ethyl 3-hydroxyhexanoate, 2305-25-1; valencene, 4630-07-3.

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Alleged Salty Taste of L-Ornithyltaurine Monohydrochloride

Tuong Huynh-ba* and Georges Philippossian

Very recently, L-ornithyltaurine hydrochloride (1) was claimed to be salty, with a saltiness equal to that of NaCl. To evaluate its organoleptic properties, peptide 1 was synthesized and rigorously purified: It was found not to be salty. The saltiness claimed earlier probably resulted from NaCl present as an artifact of the method of preparation.

Recently, several dipeptides derived from L-ornithine were described by Tada et al. (1984) to be salty. Among them, L-ornithyltaurine monohydrochloride (1) was claimed to exhibit a clear strong salty taste, equal to that of sodium chloride. Until now, most dipeptides have been reported to taste bitter, sour, sweet, slightly salty, or flat (Schiffman and Engelhard, 1976). This was the first time that a dipeptide was claimed to elicit such a strong salty taste without off-flavors. Compound 1 thus appeared to offer the greatest potential to date as salt substitute for NaCl, the excessive intake of which is considered a causative factor in certain health problems (Fregly and Kare, 1982). Many other salt substitutes have been proposed in different patent disclosures (Japan Organo K.K., 1982; Miles Laboratories Inc., 1978; Morton-Norwick Products Inc., 1974; Nisshin Oil K.K., 1982; Sterling Drug Inc., 1949), but none of them seemed to possess the property of 1. This prompted us to synthesize 1 and to perform a complete organoleptic evaluation, the results of which are here reported.

RESULTS AND DISCUSSION

Compound 1 was prepared according to the synthetic sequence described in the original work (Tada et al., 1984). Condensation of N^{α},N^{δ}-bis(benzyloxycarbonyl)-L-ornithine succinimido ester (2) with taurine (3) led to compound 4. Removal of the protective groups and subsequent acidification with HCl afforded 1 (Scheme I).

Compound 1 was tested at concentrations of 0.5% and 1%, in two separate sessions. A five-member taste panel judged compound 1 to be unsalty. A similar judgment was independently given by a trained 12-member taste panel who tested a 0.5% solution of 1. According to both panels, the off-flavors inherent to 1 were sourness, bitterness, and metallic taste, but their intensities were very low. This result was in complete disagreement with what had been claimed (Tada et al., 1984). The reason could have been the identity of the product, so our compounds 1 and 4 were fully characterized by spectroscopic means (^1H,^{13}C NMR; FAB-MS) as well as by TLC, $[\alpha]_D$ values, and elemental analyses (see the Experimental Section). In comparison, Tada et al. (1984) had assumed the purity and identity of their compounds on the basis of TLC and $[\alpha]_D$ data alone. We suspected that their compound 1 could have been contaminated by Na ion, since its immediate precursor, the sulfonic acid derivative 4, was put in contact with sodium salts (i.e., NaCl, Na_2SO_4) during its workup and would be converted to some extent into its Na salt. This property has been observed with sulfonic acid and attributed to its high acid strength (Fieser and Fieser, 1965).

Specifically to exclude all risk of contamination, we avoided the use of sodium salts in our preparation of compound 4. Compared to the original procedure (see Table I), the ethyl acetate extract containing 4 was neither washed with water saturated by NaCl nor dried over sodium sulfate.

For comparison, compound 1 was also prepared following the procedures previously described and outlined in Table I. Nevertheless, in our hands, a precipitate was formed when the ethyl acetate extract containing 4 was washed with the saturated aqueous NaCl solution and dried over Na_2SO_4. Taking this into account, we slightly modified the workup of 4 (see Table I and the Experi-

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