Table II. Concentration of THBC-3-COOH and 1-MeTHBC-3-COOH in Wine

| wine | concn, μg/mL | |
|---|---|---|
| | THBC-3-COOH | 1-MeTHBC-3-COOH |
| Ingelnook Rhine | 1.44 | 9.10 |
| Sebastiani Rhine | 1.32 | 8.32 |
| Sebastiani Chalblis | 1.09 | 5.03 |
| Gallo Chenin Blanc | ND | 1.30 |
| Sebastiani Burgundy | 0.81 | 3.72 |
| Lawrence Zinfandel | 1.70 | 6.27 |
| Cribari Chianti | 1.53 | 7.44 |

than compound 2, while the opposite is true in the wine samples; i.e., compound 2 is present in much higher concentration than 1. Previous work has shown a definite relationship between the ethanol content of the beer and the concentration of 6-OH-1-MeTHBC (Beck al., 1983); however, in the present study there does not appear to be a clear relationship between the concentration of 1 and/or 2 in beer and the ethanol content. In addition, no relationship was observed between the type of wine and the amounts of 1 and 2 found.

The origin of 1 and 2 in beer and wine is unknown and most likely is the result of both a Pictet–Spengler reaction between tryptophan and formaldehyde or acetaldehyde and their natural presence in the different brewing ingredients or grape sources. Of greater potential importance is the markedly higher levels of 1 and 2 (μg/mL) in comparison to those previously reported (ng/mL) for 1-MeTHBC (Beck and Holmstedt, 1981), 6-OHTHBC (Bosin et al., 1983), and 6-OH-1-MeTHBC (Beck et al., 1983). Since THBC compounds exhibit significant pharmacological activities (Buckholtz, 1980; Bloom et al., 1982), the presence of such high concentrations of 1 and 2 in these beverages suggests the potential for behaviorial and toxicological effects in both the normal and the alcoholic individual. The pharmacological and toxicological potential of 1 and 2 needs to be fully defined.

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Received for review January 21, 1986. Accepted May 29, 1986.

# Volatile Constituents of Greek Ouzo

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Ouzo is the most popular distilled spirit consumed in Greece. Its characteristic aroma and flavor are attributed to anethole, the main constituent of anise seed. Volatile components of two different ouzo products, Sans Rival and Callicounis, were extracted with Freon 11, centrifuged, concentrated, and analyzed by GC and GC/mass spectrometric techniques. A total of 26 components was identified. Differences in aroma and flavor between the two ouzo products are attributed to benzaldehyde and other minor components.

## INTRODUCTION

Many chemical compounds contribute to the organoleptic character of distilled spirits (Eriksson, 1978). Fatty acids, fatty acid esters, carbonyl, phenolic, sulfur, and nitrogen compounds as well as lactones, acetals, hydrocarbons, etc., have been reported to be responsible for the characteristic aroma and flavor of such spirits (Postel and Adam, 1961; Drawert and Rapp, 1965; Drawert et al., 1967; Nykänen et al., 1968; Kahn, 1969; Reinhard, 1971; Nykänen, 1972; Salo, 1973; Ronkainen et al., 1973; Nosko, 1974; Endres et al., 1976; Postel and Adam, 1976, 1977; Reinhard, 1977; Postel and Adam, 1979, 1980, 1984; Yavas and Rapp, 1985). Ouzo, the most popular distilled spirit consumed in Greece, in manufactured from the "rachis", the stem structure of the grape cluster, which are distilled.

Flavorants are then added to the distillate, the most common of which is anise seed oil. The main component comprising 80–90% of anise seed oil is anethole or 1-methoxy-4-(1-propenyl)benzene (Guenther, 1950). Other compounds identified in anise oil include (*p*-methoxyphenyl)acetone (anethole isomer), β-pinene, camphene, *d*-fenchane, dipentene, acetaldehyde, α-phellandrene, etc.

The economical importance of ouzo as both a domestic and an export product in conjunction with the fact that

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0021-8561/86/1434-0847$01.50/0 

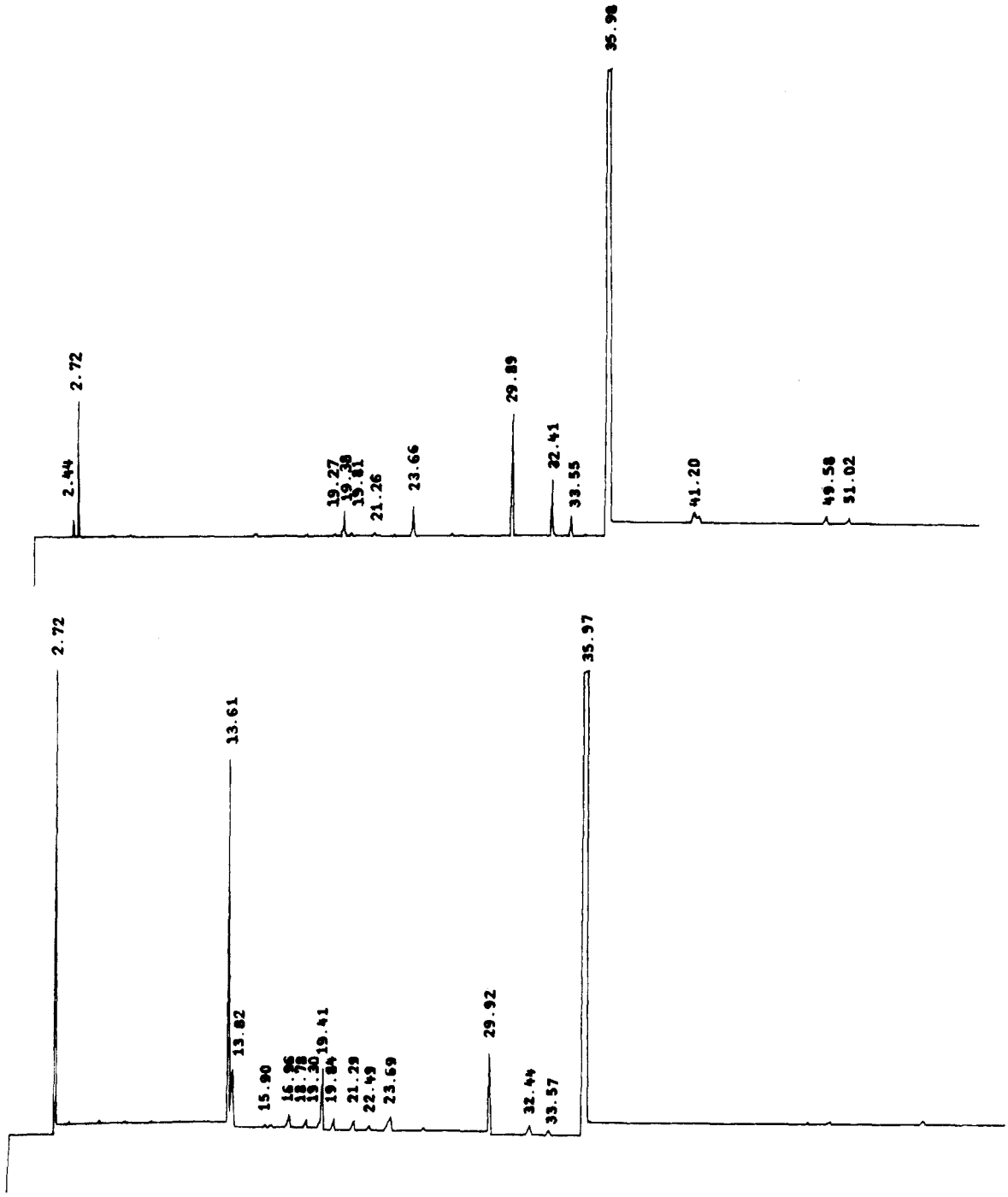


**Figure 1.** Gas chromatograms of volatile constituents of (top) Callicounis and (bottom) Sans Rival ouzo extracts.

search of the literature revealed no references on the analysis of ouzo led us to attempt to isolate and identify those compounds responsible for the characteristic flavor of ouzo.

## MATERIALS AND METHODS

Two different ouzo products originwise were studied. The first was Sans Rival ouzo, a product containing 46% v/v alcohol, produced in Attica (east central Greece) and bottled in Piraeus, Greece, and the second Callicounis ouzo, a product containing 42% v/v alcohol produced and bottled in Calamata (southern Greece).

The contents of each bottle of ouzo (750 mL) were mixed with an equal volume of water and repeatedly extracted in a separatory funnel with 150-mL volumes of distilled Freon 11 (fluorotrichloromethane). Extraction was carried out until the water/alcohol mixture was odorless. The emulsion that formed in the extract was broken by centrifugation on a Sorval refrigerated centrifuge at 10 000 rpm for 20 min at 5 °C.

The supernatent was concentrated under a slow stream of nitrogen in an analytical evaporator (Meyer N-evap, Organomation Associates Inc.). Final weight of concentrates for the Sans Rival ouzo was 2.00 g vs. 0.79 for the Callicounis ouzo.

The concentrates were analyzed on a Hewlett-Packard Model 5880 gas chromatograph equipped with a flame ionization detector. GC/mass spectrometric analyses were carried out on a Kratos MS-25 mass spectrometer connected to a Kratos DS-55 data system.

Blank runs containing distilled Freon 11 were run in parallel to samples.

GC operational conditions were as follows: column, fused silica, OV-1 50m × 0.32 mm i.d.; $T_{col}$, initial 50 °C,

**Table I. Compounds Identified in Ouzo Extract, Callicounis vs. Sans Rival[a]**

| retentn time, min | | | % area | |
|---|---|---|---|---|
| Callicounis | Sans Rival | compd | Callicounis | Sans Rival |
| 2.72 | 2.72 | Freon 11 | | |
| – | | ethyl acetate | – | * |
| | | 1,1-diethoxyethane | * | * |
| – | 13.61 | benzaldehyde | – | 3.67 |
| | 13.82 | α-pinene | * | 0.49 |
| – | 15.90 | sabinene | – | 0.03 |
| | | β-pinene | * | * |
| – | 16.96 | myrcene | – | 0.10 |
| | 18.78 | *p*-cymene | * | 0.07 |
| 19.27 | 19.30 | 1,8-cineole | 0.06 | 0.07 |
| 19.38 | 19.41 | limonene | 0.29 | 0.72 |
| 19.81 | 19.84 | β-ocimene | 0.06 | 0.11 |
| 21.26 | 21.29 | γ-terpinene | 0.04 | 0.09 |
| | 22.49 | fenchone | * | 0.04 |
| 23.66 | 23.69 | linalool | 0.36 | 0.19 |
| | | camphor | * | * |
| | – | 4-terpineol | * | – |
| | – | α-terpineol | * | – |
| 29.89 | 29.92 | estragole | 1.66 | 1.06 |
| – | | benzaldehyde, diethylacetal | – | * |
| 32.41 | 32.44 | *p*-anisaldehyde | 0.73 | 0.10 |
| 33.55 | 33.57 | anethole isomer | 0.27 | 0.07 |
| 35.98 | 35.97 | anethole | 95.90 | 93.18 |
| 41.20 | – | anisylacetone (T) | 0.10 | – |
| – | | γ-caryophyllene (T) | – | * |
| – | | β-caryophyllene | – | * |
| 49.58 | – | sesquiterpene | 0.08 | – |
| | | | 99.55 | 99.99 |

[a] – = not identified; * = trace component; T = tentative.

final 225 °C, rate 2 °C/min; $T_{det}$, 260 °C; $T_{inj}$, 250 °C; carrier gas, helium, rate 1.8 mL/min; sample size, 0.1 μL; split ratio, approximately 100:1.

mass spectral operation conditions were as follows: electrovoltage, 70 eV; acceleration voltage, 2 kV; scanning rate, 0.7 s/decade; *T*(ion source), 170 °C; split ratio, approximately 100:1.

## RESULTS AND DISCUSSION

Compounds identified, retention times, and relative areas for each compound for both ouzo products are shown in Table I. Comparison of the GC profiles of the two products is given in Figure 1. Compounds were identified on the basis of mass spectra by comparison to library mass spectral data. Blank runs gave only one peak at tr = 2.72 min corresponding to Freon 11.

Table I reveals moderate differences between these two products. The major difference being the presence of benzaldehyde in the Sans Rival product. Synthetic benzaldehyde is probably being added along with other natural flavorants. There is also a slight decrease in the concentration of estragole (1.66%) in the Callicounis vs. (1.06%) in the Sans Rival product, α-pinene (trace vs. 0.49%), *p*-anisaldehyde (0.73% vs. 0.10%), and limonene (0.29% vs. 0.72%) as well as in the concentration of anethole (95.90% vs. 93.18%).

These differences probably result from the use of different flavoring materials and the addition of different amounts of these materials by local manufactures.

## ACKNOWLEDGMENT

I thank International Flavors and Fragrances Inc. for the use of their instrumentation and P. Giannakakos for his technical assistance.

**Registry No.** PhCHO, 100-52-7; α-pinene, 80-56-8; sabinene, 3387-41-5; myrcene, 123-35-3; *p*-cymene, 99-87-6; 1,8-cineole, 470-82-6; limonene, 138-86-3; β-ocimene, 13877-91-3; γ-terpinene, 99-85-4; fenchone, 1195-79-5; linalool, 78-70-6; estragole, 140-67-0; *p*-anisaldehyde, 123-11-5; anethole isomer (*p*-$MeOC_6H_4CH_2Ac$), 122-84-9; anethole, 104-46-1; anisylacetone, 104-20-1.

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Received for review May 29, 1984. Revised manuscript received November 8, 1985. Accepted April 9, 1986.