Identification of 2,4,6-Trichloroanisole as a Potent Compound Causing Cork Taint in Wine

Hans-Rudolf Buser,* Carla Zanier, and Hans Tanner

By use of gas chromatography–mass spectrometry, 2,4,6-trichloroanisole (2,4,6-TCA) was identified in bottled and corked European grape wines as the main component responsible for the musty cork taint occasionally associated with faulty wines. Concentrations of 2,4,6-TCA found in a series of red and white wines with this distinct off-flavor ranged from 20 to 370 ppt (ng/L). In wines of good organoleptic quality, this compound was not detectable (<2–8 ppt). Organoleptic tests showed 2,4,6-TCA to be a very potent compound perceivable in wine at concentrations as low as 10 ppt. It is presumed that 2,4,6-TCA and other related chlorinated compounds originate from chlorination of lignin-related substances during the chlorine bleaching used in the processing of cork and that these compounds are later extracted into the wine.

Wines with cork taint have posed a problem in the past and apparently have been becoming more common during recent years (Charpentier, 1977; Pes and Vodret, 1971; Schaeffer et al., 1978). The unpleasant, musty off-flavor can render an otherwise excellent wine completely useless and may cause economic loss. It is a feared and serious problem for both wine producers and wine tasters. The phenomenon can be observed with wines from various locations, and it does not seem to be restricted to any special type, variety, or brand of wine. The reasons for this cork taint have so far eluded attempts to trace its origin (Perscheid, 1976; Zanier and Tanner, 1979). Although more than 140 flavor components in wine were identified (Drawert and Rapp, 1968), none could account for this musty off-flavor.

In the present investigation we report the identification of 2,4,6-trichloroanisole (2,4,6-TCA) as the main component responsible for this cork taint. Using high-resolution gas chromatography with direct odor characterization and combined with mass spectrometry, we found that ppt (parts per trillion) concentrations of this compound are responsible for this off-flavor. To our knowledge, this is the first report on the occurrence of 2,4,6-TCA in wine and the identification of this compound as the cause for cork taint. 2,4,6-TCA, however, has been previously identified as an off-flavor component in eggs and chicken broilers (Bemelmans and ten Noever de Brauw, 1974). In that case, the chemical was traced to chlorophenols contaminating feed and litter with 2,4,6-TCA formed through microbial action. In our case, we presume that 2,4,6-TCA originates from the chlorination of lignin-related compounds during chlorine bleaching in the processing of cork.

EXPERIMENTAL SECTION

Wines Investigated. A series of red and white wines, some with a distinct cork taint, were obtained from commercial sources. The bottled and corked wines were Chianti Classico and Refosco from Italy, Rioja from Spain, Dorin and Riesling-Sylvaner from Switzerland, and Ruländer from Germany. If possible, wines of good organoleptic quality and of the same provenance, variety, and brand as the cork-tainted wines were obtained and analyzed at the same time.

Extraction and Sample Preparation. In preliminary analyses, portions of 500 mL of wine were extracted 3 times with 50 mL of pentane–ethyl acetate (3:1) in a separatory funnel, and the combined extracts were concentrated to 1–2 mL and partitioned with 2 mL of 1 N NaOH to remove interfering acidic components. The organic phase was carefully concentrated to 0.2 mL in a stream of nitrogen. An aliquot of 2 μL was analyzed.

A modified extraction procedure was employed for analysis of a larger series of samples. Portions of 100 mL of wine (cooled to 4 °C) were extracted in a narrow-neck 100-mL volumetric flask 3 times with 2 mL of pentane. The organic phase was removed with a pipet and placed onto a prewashed silica gel minicolumn (0.5 g of silica gel; 70–230 mesh; Merck; 140 × 5 mm disposable Pasteur pipet). The components of interest were eluted with a total of 8–10 mL of pentane. The eluate was carefully concentrated to 100 μL, and an aliquot of 1–2 μL was used for analysis. Extreme care was taken to never let the extracts go to dryness as 2,4,6-TCA is easily lost.

Additionally, the cork of a faulty Chianti wine was analyzed. The cork was cut into small pieces (2–3 mm) and extracted with 10–15 mL of pentane–ethyl acetate. The extract was decanted, the organic phase was concentrated, insolubles were removed, and an aliquot was analyzed.

Recovery experiments were carried out by adding known quantities of 2,4,6-TCA to wine, followed by extraction and analysis.

Reference Compounds. Chloroanisoles were prepared through methylation with diazomethane of commercial chlorophenols (Fluka AG, Buchs, Switzerland). Standard solutions in ethanol were prepared at concentrations of 50 and 1 ng/μL. These solutions were used for spiking wines for analytical and organoleptic tests. Solutions in *n*-hexane were prepared at concentrations of 100 pg/μL for gas chromatographic and mass spectrometric analyses.

Gas Chromatographic (GC) Analyses. A 25-m Ucon 50 HB 5100 (0.3-mm i.d.) fused silica capillary column was installed in a Carlo Erba GI gas chromatograph with flame ionization detection (FID). Aliquots of 1–2 μL of sample extracts were injected splitlessly (30 s) with the column at 30 °C; the column temperature was then programmed at 3 °C/min to 200 °C.

In additional analyses, odor evaluations of components eluting from the capillary column were carried out. Care was taken to ensure identical GC conditions as when using FID. Odor characterizations were made by sniffing at the FID nozzle with the flame shut-off.

Gas Chromatographic–Mass Spectrometric (GC–MS) Analysis. The Ucon 50 HB 5100 fused silica column was coupled to a Finnigan 4000 quadrupole mass spectrometer with a 6115 data system. The column led directly into the ion source and as such no interface was required. Samples were analyzed with the MS operating in the electron impact (EI, 70 eV) or chemical ionization (CI,

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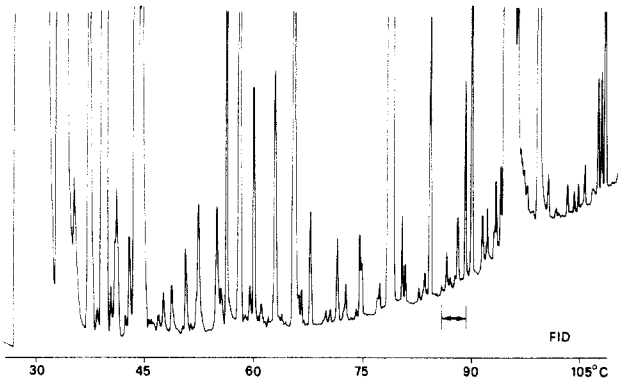

Figure 1. FID gas chromatogram of the extract of cork-tainted Chianti Classico (sample 2) showing elution of a multitude of components from the 25-m Ucon 50 HB 5100 high-resolution column. The elution of the cork-taint component in the region (86–89 °C) is indicated by arrows.

iso-butane) mode. Mass spectra were recorded by repetitive scanning using the data system (EI, m/z 35–385; CI, m/z 85–435; 1.35 s/scan).

Trace analyses for 2,4,6-TCA were carried out by selected ion monitoring (mass fragmentography) using molecular ions at m/z 210 and 212 (EI mode). Quantifications were made by comparison of peak heights of a sample to those obtained for known quantities of the reference compound (external standard procedure). The minimal detectable quantity of 2,4,6-TCA was 2–5 pg, corresponding to concentrations of 2–5 ppt.

Organoleptic Tests. Wines of good organoleptic quality were selected and fortified with 2,4,6-TCA at the 0, 10, 30, and 100 ppt (ng/L) level. Organoleptic tests were carried out by a panel of experienced wine tasters given samples in a blind fashion.

RESULTS AND DISCUSSION

In a preliminary study, two Chianti Classico wines (1978; origin Italy) were analyzed. Whereas one of the wines was of good organoleptic quality, the other had a distinct cork taint. Both Chiantis were of the same brand and year. They were extracted and acidic components removed since they interfered with subsequent GC analyses. The musty cork taint was found to remain in the neutral fraction.

A multitude of components was observed in FID chromatograms of the extracts by using a Ucon 50 HB 5100 high-resolution capillary column. The chromatogram (see Figure 1) of the cork-tainted Chianti Classico was almost indistinguishable from that of the good-quality wine. Odor characterization of eluting components, however, revealed the elution of a cork-taint component from the faulty wine at an elution temperature of 86–89 °C as indicated in Figure 1. The elution of this component was not observed with the good-quality Chianti Classico.

GC analyses of a set of Rioja wines (1979; origin Spain) gave a similar finding. Again, the FID chromatogram of the cork-tainted wine was indistinguishable from that of the good quality wine, and again odor characterization revealed the elution of a cork-taint component associated only with the faulty wine. The retention time of the Rioja cork-taint component was the same as that of the Chianti component. With both cork-tainted wines, this component was found to elute at about midrange between ethyl phenylacetate and 2-phenylethyl acetate. However, no significant difference could still be observed in the FID chromatograms at these locations for the good-quality and the cork-tainted wines.

Extracts of these wines were analyzed by GC–MS using EI and CI. In Table I we list the compounds identified

Table I. Major Compounds Identified in the Elution Range of the Cork-Taint Component in Chianti Classico and Rioja (Samples 1-4)

peak no.[a]	compound	M_r[b]	mass spectral data, m/z[c] EI	CI
1	diethyl butanedioate	174	174, 147, 129, 101	175
2	α-terpineol	154	139, 136, 121, 93, 59	137
3	ethyl decanoate	200	200, 157, 155, 101, 88	201
4	1-phenylethanol	122	122, 107, 79, 77	105
5	ethyl phenylacetate	164	164, 91	165
6	ethyl nicotinoate	151	151, 123, 106, 78	152
7	ethyl decenoate	198	152, 101, 88	199
8	2-methylnaphthalene	142	142, 141, 115	143
9	2-phenylethyl acetate	164	104, 91	165
10	phenylmethanol	108	108, 107, 79, 77	147
11	diethyl pentanedioate	188	143, 142, 115, 101, 87	189
12	benzothiazole	135	135, 108	136
13	2-phenylethanol	122	122, 92, 91	105

[a] Peak numbers as used in Figure 2. [b] Molecular weight. [c] Major ions observed in the mass spectrum.

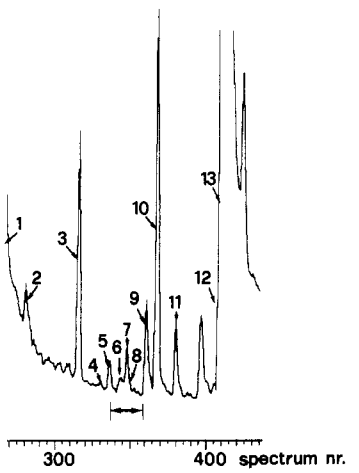

Figure 2. Partial computer reconstructed chromatogram of the extract of cork-tainted Chianti Classico (sample 2). Total ion chromatogram (EI, m/z 35–385; 1.35 s/scan); the elution of the cork-taint component was in the region of the spectrum 336–359; for peak identifications, see Table I.

that elute in the region of interest, and a partial computer reconstructed chromatogram (total ion, EI mode) for one of the samples is shown in Figure 2. The same compounds were found in all four wines analyzed and at similar concentrations in both sets of wines. These compounds were judged not to be essential for the cork taint. Several additional compounds were found to be present but could not be identified at that time. However, these compounds too were found to be present in all four wines at similar concentrations and thus could not be responsible for the cork taint. These results indicated that the cork-taint component had to be present at an extremely low concentration.

At this point an extract of the cork of the cork-tainted Chianti Classico was analyzed. GC analysis and odor characterization revealed the presence of the same cork-taint component (elution temperature of 86–89 °C) as in the cork-tainted wines. GC–MS analysis of the cork extract revealed the presence of the same compounds as listed in Table I, but additionally several chlorinated compounds were also detected, one of which was eluting

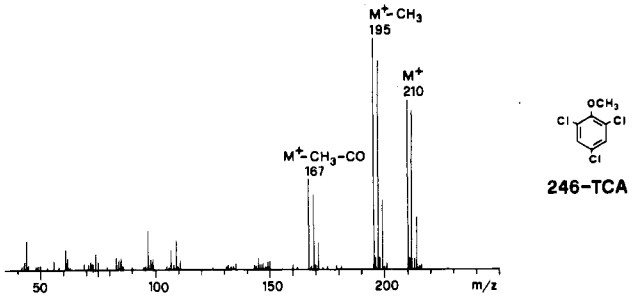

Figure 3. Mass spectrum (EI) of the chlorinated compound identified as 2,4,6-TCA in the extract of the cork of cork-tainted Chianti Classico (sample 2).

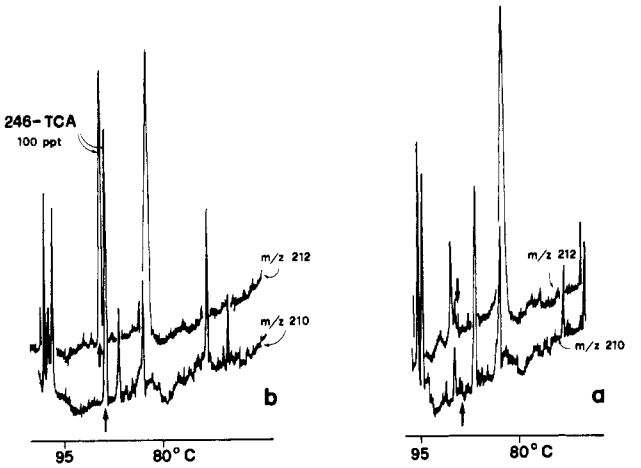

Figure 4. Mass fragmentograms (EI, m/z 210 and 212) showing (a) the absence of 2,4,6-TCA (<5 ppt) in the extract of good-quality Chianti Classico (sample 1) and (b) the presence of 100 ppt of 2,4,6-TCA in the extract of cork-tainted Chianti Classico (sample 2). For experimental conditions, see the text.

at the expected retention time of our unknown cork-taint component. The mass spectrum of this chlorinated compound indicated a molecular ion at m/z 210 and the presence of three chlorine atoms (see Figure 3). Fragmentation to $M^+ - 15$ and $M^+ - 43$ is indicative of aromatic methyl ethers ($M^+ - CH_3$; $M^+ - CH_3 - CO$). Subsequently the compound was identified as a trichloroanisole by comparison of the mass spectrum of the unknown compound to that of reference compounds. Careful inspection and computer searching of the GC–MS data of these wine extracts revealed the presence of a trichloroanisole only in case of the cork-tainted Chianti Classico (sample 2) (low-intensity ions with chlorine clusters at m/z 210, 195, and 167). Obviously, the concentration of this compound in the other wine extracts was too low to allow detection by mass spectrometry.

Samples 1–4 were reanalyzed for the presence of trichloroanisole by selected ion monitoring (mass fragmentography) using the molecular ions at m/z 210 and 212. Mass fragmentograms of the two Chianti Classicos are shown in Figure 4. The cork-tainted wine (sample 2) revealed the presence of trichloroanisole at a level of 100 ppt. In the good-quality Chianti (sample 1), this compound was not detectable (<5 ppt). Similar chromatograms and results were obtained for the two Rioja wines: trichloroanisole at a level of 60 ppt was detected in the cork-tainted wine (sample 4) but not in the good quality wine (sample 3). The results are summarized in Table II. The presence of trichloroanisole was confirmed by the correct response ratio between the ions at m/z 210 and 212. The trichloroanisole was identified as the 2,4,6-substituted isomer (2,4,6-TCA) by comparison of its retention time on the Ucon 50 HB 5100 capillary column to those of reference compounds. The other isomeric trichloroanisoles (2,3,4-, 2,3,5-, 2,3,6-, 2,4,5- and 3,4,5-TCA) were all separated from the first eluting 2,4,6-TCA under the experimental conditions used.

Table II. Concentration of 2,4,6-TCA in Various Wines As Determined by Mass Fragmentography

sample	variety of wine (provenance)[a]	organoleptical quality	2,4,6-TCA,[b] ppt
1	Chianti Classico, 1978a (Italy)	good	<5
2	Chianti Classico, 1978a (Italy)	cork taint	100
3	Rioja, 1979b (Spain)	good	<6
4	Rioja, 1979b (Spain)	cork taint	60
5	Chianti Classico, 1979 (Italy)	good	<5
6	Chianti Classico, 1974 (Italy)	good	<2
7	Rioja, 1973 (Spain)	good	<2
8	Rioja, 1978c (Spain)	good	<8
9	Rioja, 1978c (Spain)	cork taint	27
10	Refosco, 1977 (Italy)	strong cork taint	370
11	Dorin, 1979d (Switzerland)	good	<5
12	Dorin, 1979d (Switzerland)	cork taint	33
13	Dorin, 1979d (Switzerland)	cork taint	22
14	Dorin, 1979d (Switzerland)	musty	<3
15	Riesling-Sylvaner, 1974 (Switzerland)	good	<1
16	Ruländer, 1979 (Germany)	cork taint	73

[a] Identical lettering was used to identify sets of wines (same type, brand, and year). [b] Results not corrected for recovery values.

Table III. Recovery Values of 2,4,6-TCA Added to Wine prior to Extraction and Cleanup

wine (sample)[a]	2,4,6-TCA fortification, ppt	2,4,6-TCA found, ppt	recovery, %
Chianti Classico (1)	100	72	72
Chianti Classico (6)	30	13	43
Rioja (7)	70	39	56
Riesling-Sylvaner (15)	10	7	70
Riesling-Sylvaner (15)	100	58	58

[a] Sample numbering refers to that in Table II.

Small portions of extract of the good-quality Chianti and Rioja (samples 1 and 3, respectively) were fortified with 2,4,6-TCA at a level corresponding to 100 ppt in the original wine. GC analysis and odor characterization of eluting components now revealed the typical cork taint at the expected retention time.

An additional number of wines (samples 5–16, Table II) were analyzed for 2,4,6-TCA by using the simplified extraction procedure and selected ion monitoring. The results are reported in Table II. In good-quality red wines (samples 5–8), 2,4,6-TCA was not detectable (<2–8 ppt). However, two cork-tainted red wines (samples 9 and 10) showed the presence of 2,4,6-TCA at a concentration of 27 and 370 ppt, respectively. In good-quality white wines (samples 11 and 15), 2,4,6-TCA was not detectable (<1–5 ppt). However, this compound was again detected in the cork-tainted wines (samples 12, 13, and 16) at concentrations of 33, 22, and 73 ppt, respectively. In a musty wine (sample 14; bottle closed by a screw cap), 2,4,6-TCA was not found to be present (<3 ppt).

Recovery experiments were carried out by adding known amounts of 2,4,6-TCA to some good-quality red and white wines. The results shown in Table III indicate recovery rates ranging from 43 to 72% at levels ranging from 10 to 100 ppt of 2,4,6-TCA in wine.

In preliminary organoleptic tests, red and white wines of excellent quality were fortified with 2,4,6-TCA at the

0-, 10-, 30-, and 100-ppt level and submitted to a panel of 10 tasters in a blind fashion. The tasters were asked to compare the fortified samples to reference samples of the original wines. All persons recognized a cork taint in wines spiked at the 100-ppt level. An off-flavor was still recognized at the 30- and 10-ppt level by nine and five persons, respectively. These preliminary results indicate the extreme potency of 2,4,6-TCA and show that concentrations as low as 10 ppt can be perceived organoleptically and thus diminish the quality of wine. The actual amounts of 2,4,6-TCA recognizable by the human nose apparently are as low as a few picograms (10^{-12} g).

Additional chlorinated compounds were detected in treated cork by using GC–MS. They included dichloroanisole, mono-, di-, and trichlorophenol, mono- and dichloromethoxyphenol (guaiacols), di- and trichlorodimethoxybenzene (veratroles), and chloronaphthol. The significance of all these compounds for the cork taint in wine remains to be investigated.

CONCLUSIONS

2,4,6-TCA was found as a major component responsible for the musty cork taint occasionally found in cork-bottled wines. Cork taint in wine was caused from concentrations of 2,4,6-TCA in the ppt range and proved the extreme potency of this compound. Although 2,4,6-TCA could be perceived with ease by the human nose at these extremely low concentrations, the detection and identification required sophisticated and extremely sensitive analytical techniques such as high-resolution gas chromatography and mass spectrometry. The presence of 2,4,6-TCA at these small levels in wine very likely poses no toxicological risk; nevertheless, it completely destroys the quality of this product.

The origin of 2,4,6-TCA in wine is not yet fully known. Because larger quantities of this and related chlorinated compounds (chlorinated anisols, phenols, guaiacols, and veratroles) were detected in cork used for bottling of wine, the involvement of this material is strongly suggested. The occurrence of 2,4,6-TCA and these related compounds could possibly arise from the chlorination of lignin-related compounds during chlorine bleaching used in the processing of cork and later extraction of these compounds into the wine. If this proves to be true, replacement of chlorine treatment in the processing of cork should remedy the cork-taint problem.

In further experiments, the occurrence of 2,4,6-TCA and the other extraneous compounds should be documented. Simplified analytical methods (e.g., electron-capture detection of 2,4,6-TCA) could prove to be suitable for this purpose. Such analyses could supplement but hardly replace organoleptic tests. We believe that finding 2,4,6-TCA as a major cause for cork taint in wine is an important contribution in continuing efforts to maintain the quality and purity of this product.

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2-Acetyl-5-chloropyrrole in the Volatile Flavor Constituents of Cocoa Butter

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The presence of 2-acetyl-5-chloropyrrole in the volatile flavor constituents of cocoa butter was confirmed through synthesis of the authentic compound. This compound was synthesized by chlorination of 2-acetylpyrrole. The structure of the synthesized compound was established by infrared, nuclear magnetic resonance, and mass spectrometry. The identification of this compound in the volatile flavor constituents of cocoa butter was confirmed by comparing the mass spectrum and gas chromatographic retention time with those of the authentic sample. 2-Acetyl-5-chloropyrrole is the first chlorinated heterocyclic compound identified in the volatile flavor of foods.

The isolation and identification of the volatile flavor constituents from cocoa butter have been recently described (Ho et al., 1981). The present paper reports confirmation of the presence of 2-acetyl-5-chloropyrrole through synthesis of the authentic compound.

The presence of halogenated aliphatic and aromatic hydrocarbons has been reported in the volatiles of various foods, such as baked potatoes (Ho and Coleman, 1981), canned beef stew (Chang and Peterson, 1977), vinegar (Kahn et al., 1972), and cheeses (Dumont et al., 1974a,b). These halogenated hydrocarbons may be undesirable to human health (Fishbein, 1979a,b). Heterocyclic compounds are widely distributed in food aromas. However, no halogenated heterocyclic compounds have been identified.

The synthesis of 2-acetyl-4-chloropyrrole and 2-acetyl-5-chloropyrrole was undertaken in order to determine the exact structure of a monochloroine-substituted acetylpyrrole in the volatile flavor constituents of cocoa butter.

EXPERIMENTAL SECTION

Synthesis of 2-Acetyl-5-chloropyrrole and 2-Acetyl-4-chloropyrrole. 2-Acetylpyrrole was purchased

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