2; butyl hexahydrophthalide, 3553-34-2; (*Z*)-butylidenephthalide, 72917-31-8; cnidilide, 3674-03-1; (*Z*)-ligustilide, 81944-09-4; butylphthalide, 6066-49-5; *trans*-neocnidilide, 3553-29-5; *cis*-neocnidilide, 4567-33-3; senkyunolide, 63038-10-8; (*E*)-ligustilide, 81944-08-3; nitrate, 14797-55-8.

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Analytical Investigation of Rio Off-Flavor in Green Coffee

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About 20% of Brazil coffee production presents the so-called Rio defect characterized by a strong off-flavor, often described as medicinal, phenolic, or iodine-like. Occasionally, this defect also occurs in coffees from other origins. An extensive investigation was carried out to identify the compound(s) responsible for the coffee samples. Volatiles were isolated from green beans by simultaneous distillation–extraction and analyzed by capillary GC, GC–sniffing, and GC–MS. 2,4,6-Trichloroanisole (TCA) was identified as the most likely key compound for the Rio off-flavor. TCA was found in all Rio samples in concentrations ranging from 1 to 100 ppb. Less than 50% of TCA present in green beans was lost during roasting. 2,4,6-Trichlorophenol (TCP), the probable precursor of TCA, was also found in most of these samples. Adding TCA to freshly brewed coffee imparted to it the same off-flavor notes as described in actual Rio coffee. The perception threshold of TCA in coffee brew was found to be 8 ppt for direct odor perception and 1–2 ppt for flavor by mouth.

Brazilian coffees are classified according to the quality of the beverage obtained after roasting and brewing. The categories range from "soft", for the best samples, to "hard" and "rio", for samples with increasing harshness (Jobin, 1982). Rio flavor is a pronounced harsh taste mainly found in coffees from the State of Rio, Minas Gerais, Zona da Mata, and the northwest of Esperito Santo. The associated olfactive note has been described alternatively as medicinal, phenolic, or iodine-like. According to our experience, however, the odor of strong Rio coffee is better depicted as a musty, cellarlike odor. This characteristic flavor, considered as an off-flavor by a majority of the consumers, develops more particularly when coffee is harvested under wet climate conditions and affects 20–25% of Brazilian coffees. Occasionally, Rio flavor has also been found in coffees grown in other parts of the world.

Although Rio coffee has been known for generations, knowledge on the origin and the cause of this defect is still quite limited. However, it was suspected to be the consequence of some kind of fermentation. Amorim et al. (1979) related it to the action of endogenous polyphenol oxidase and peroxidase triggered by structural changes of bean cell membranes. More recently, however, microscopic and microbiological investigations undertaken in our laboratories revealed that Rio beans were heavily infested with various fungi (Aspergilli, Fusaria, Penicillia, Rhizopus, etc.) and bacteria (Lactobacilli, Streptococci), resulting in a more or less complete degradation of the cell structure (Dentan, 1988; Vanos, 1988).

We have also searched for the components responsible for the characteristic Rio off-flavor. Volatiles from healthy and Rio green coffees were isolated and analyzed by gas chromatography combined with flame ionization, mass spectrometry, and odor detection. In the

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first part of this study, 2,4,6-trichloroanisole (TCA) was identified as being an important contributor to the Rio flavor (Spadone and Liardon, 1988). A more detailed investigation was then undertaken to confirm this and to determine whether other compounds were involved in the off-flavor.

The results of the complete study are presented here. They confirm the major role of TCA in Rio off-flavor and give some clues as to the possible origin of this compound in green coffee.

EXPERIMENTAL SECTION

Rio Coffee Samples. Nine samples of green coffees with a more or less pronounced Rio flavor were obtained from Brazil and Puerto Rico (list in Table V). The strongest Rio flavor was observed in Puerto Rico A sample. This coffee also presented the flavor and appearance characteristics of other kinds of degradation, including fermentation. A sample of green coffee (Santos control), free of any off-flavor, was used as a reference.

Samples of two Rio coffees (Puerto Rico A and Santos Rio) and of the reference were roasted to 18% weight loss (medium roast) in a laboratory-scale roaster.

Coffee Extraction. Green coffee volatiles were isolated by simultaneous distillation–extraction (SDE). A 40-g sample of green beans was immersed in liquid nitrogen, then finely ground in an electric coffee mill, and added to 250 mL of distilled water. The resulting suspension was placed in a modified Nickerson–Likens apparatus (Schultz et al., 1977) and extracted for 3 h with 60 mL of dichloromethane. With the exception of the freezing step, the same procedure was applied to roasted coffees.

For the quantitative determination of polychlorophenols, the ground coffee suspension was adjusted to pH 2 by the addition of 2N HCl before the extraction. This procedure was only introduced at a later stage of the study, while some of the Rio coffee samples were no longer available.

After being dried over anhydrous sodium sulfate, the organic extract was concentrated in two steps: from 60 to 1–3 mL by distillation, on a Widmer column, and then to about 200 μL by evaporation under a gentle stream of nitrogen. Before this second step 50 μg of BHT (2,6-di-*tert*-butyl-1-hydroxy-4-methylbenzene) was added to serve as an internal standard. Concentrated extracts were kept at −20 °C until analysis.

Derivatization of Chlorophenols. Chlorophenols were analyzed after conversion into chlorophenetoles according to the procedure of Gee and Peel (1974). Following the distillation step, diazoethane was added to the organic extract until persistence of a pale yellow color and allowed to react for 30 min at room temperature. BHT was then added to serve as an internal standard, and the sample was further concentrated to about 0.2 mL under a gentle stream of nitrogen.

Gas Chromatography (GC). Preliminary investigations were carried out on an Hewlett-Packard (HP) 5710 gas chromatograph with a flame ionization detector (FID), equipped with a 30 m × 0.25 mm (i.d.) DB-WAX (J&W Scientific) fused-silica capillary column (film thickness 0.25 μm). Injections were done in the split mode (split ratio 20:1, sample size 1 μL). The injector temperature was 250 °C, and the oven temperature was programmed from 20 to 210 °C at 4 °C/min. Hydrogen was used as the carrier gas.

Alternatively, the same instrument was equipped with a 30 m × 0.32 mm (i.d.) DB-5 fused-silica capillary column (film thickness 0.25 μm). A retention gap, consisting of 1 m of fused-silica tubing (0.32-mm i.d.), was attached to the front of the column with a press-fit connector (MSP Friedli & Co, Koeniz, Switzerland). On-column injection was used. The column effluent was split 1:1 (via an all-glass press-fit splitter) to the FID and a heated outlet port (200 °C). The port effluent was either sniffed or collected for further analyses. For sniffing, the effluent was mixed with a 50 mL/min humidified air flow. Fraction collection was made in short sections of deactivated fused-silica tubing (30 cm × 0.32 mm (i.d.)) and cooled in a Dewar flask filled with liquid nitrogen.

Trapped fractions were reanalyzed on a 30 m × 0.32 mm (i.d.) DB-WAX fused-silica capillary column by attaching the traps to the column (off-line bidimensional GC). The column effluent was directed to an FID, or to a sniffing port. The oven temperature was programmed from 40 to 180 °C at 4 °C/min.

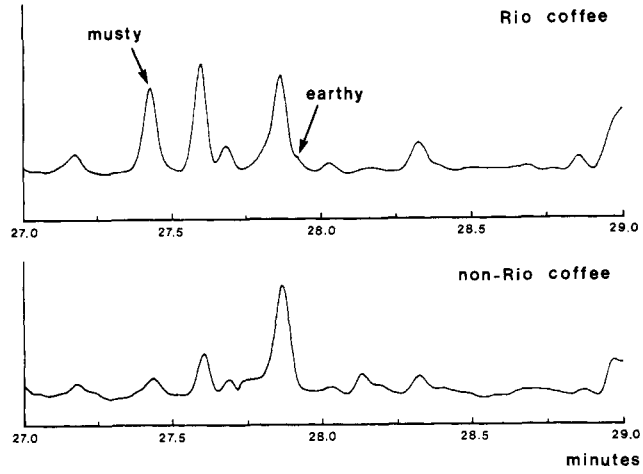

Figure 1. Partial chromatogram of green coffee extracts showing the location of Rio-like smelling compounds: top, Rio coffee; bottom, reference coffee. For analytical conditions, see text.

Table I. Components Identified in the Partial Chromatogram[a] of a Reference and a Rio Coffee

ret time, min	Rio coffee	ref coffee
27.4	ethyl salicylate 2,4,6-trichloroanisole	ethyl salicylate
27.8	unknown geosmin	unknown

[a] Reproduced in Figure 1.

Gas Chromatography–Mass Spectrometry (GC–MS). Coffee extracts were analyzed on HP 5992 and 5995 GC/MS systems, alternatively equipped with a 30 m × 0.25 mm (i.d.) DB-WAX fused-silica capillary column (temperature programmed from 20 to 210 °C at 4 °C/min) or with a 50 m × 0.32 mm HP-5 fused-silica capillary column (temperature programmed from 20 to 230 °C at 4 °C/min). Trapped fractions were anlayzed on an HP 5890 gas chromatograph interfaced to a Finnigan MAT 8430 mass spectrometer. The column was a 25 m × 0.32 mm (i.d.) Carbowax 20M fused-silica capillary column prepared according to Traitler (1983). Column temperature was programmed from 40 to 180 °C at 4 °C/min. A second set of trapped fractions was run on a Finnigan MAT 4000 GC–MS–INCOS system, equipped with a 50 m × 0.32 mm (i.d.) Carbowax 20M fused-silica capillary column. The oven temperature was programmed from 40 to 200 °C at 4 °C/min. All instruments were operated in the electron-impact mode at an ionization voltage of 70 eV. Helium was used as the carrier gas in all cases.

Quantitative Analysis of Chlorophenols and Chloroanisoles. For quantification, BHT served as an internal standard. Linear calibration curves were obtained for all compounds in the 0.5–50 ppb range. The following values were found for the recovery yield of the various phenols and anisoles in green coffee suspensions by SDE: 2,4,5- and 2,4,6-trichlorophenol (TCP), 70–76%; 2,3,5,6-tetrachlorophenol (TeCP), 35%; pentachlorophenol (PCP), 18%; 2,4,5- and 2,4,6-trichloroanisole (TCA), 79–83%; pentachloroanisole (PCA), 90%. The concentrations of chlorophenols and chloroanisoles in green and roast coffee extracts were determined with use of GC–MS in the selected ion monitoring mode (SIM). The analyses were performed on an HP 5992 GC–MS system under the same conditions as described in the previous section. Chlorophenols were determined in samples after derivatization with diazoethane. On the basis of mass spectra of authentic compounds, the following ions were monitored: TCP, *m/z* 196, 198, 224; TeCP, *m/z* 230, 232, 258; PCP, *m/z* 264, 266, 292; TCA, *m/z* 195, 197, 210; TeCA, *m/z* 229, 231, 244; PCA, *m/z* 265, 267, 280; BHT, *m/z* 205, 220.

Sensory Perception Threshold of TCA. Absolute odor threshold of TCA was determined by GC–sniffing. The chro-

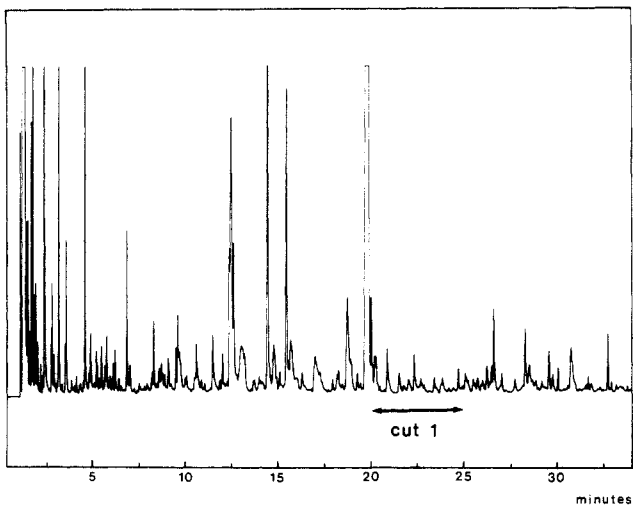

Figure 2. Chromatogram of Puerto Rico A coffee (Rio coffee) extract analyzed on a 30 m × 0.3 mm DB-5 fused-silica capillary column. Cut 1 indicates the region trapped and reanalyzed on a polar capillary column (see Figure 4).

matograph was equipped with a 30 m × 0.25 mm (i.d.) DB-5 fused-silica capillary column. The carrier gas was hydrogen at 0.8 bar, and the column temperature was programmed from 80 to 250 °C at 16 °C/min. Stepwise dilutions of TCA in isooctane were injected on-column (1 μL) until no odor could be perceived at the TCA retention time. TCA odor detection was performed independently by three assessors, and each dilution was tested two or three times. Replicate determinations were made on different days to avoid saturation effects. The lowest detectable amount (LDA) was defined as the amount for which a positive response was achieved in 50% of the cases.

TCA odor and taste threshold in coffee brew was determined by adding known amounts of TCA in the form of alcoholic solutions to freshly brewed coffee, prepared with a filter machine from 50 g of ground coffee/L of water. On the basis of a first screening to determine the order of magnitude of the

Table II. Odorgram of Puerto Rico A Extract at 243× Dilution[a]

ret time, min	ret ind	odor note	component
4.70	870	potato	
5.45	896	nutty	
6.46	933	unidentified	
6.67	940	sulfur	
6.97	950	unidentified	
7.30	960	mushroom	
9.86	1034	unpleasant, slightly earthy	
10.72	1059	sow bug	
11.45	1078	cucumber, green	
11.75	1085	floral	
13.07	1122	cucumber	
13.52	1135	cucumber	2-nonenal
14.46	1161	bell pepper	2-methoxy-3-isobutylpyrazine
14.74	1168	sulfur	
17.15	1235	oats	
18.61	1277	cloves	4-vinylguaiacol
19.17	1291	musty	2,4,6-trichloroanisole
20.57	1334	straw	
21.23	1354	tobacco	
21.86	1373	earthy	geosmin

[a] Chromatogram reproduced in Figure 2.

perception thresholds, coffee samples were assayed with TCA concentrations ranging between 1 and 10 ng/L for direct olfactive perception and 0.5 and 2 ng/L for oral perception. Samples were presented in a triangle test, each triangle comprising two neutral coffees and one spiked with TCA. Tasters were asked to identify the spiked coffee and to describe the off-note. Thresholds were defined as the 50% perception concentration. Sixty-milliliter samples of coffee at 60 °C were used for odor and taste threshold. For direct olfactive perception, the samples were presented in preheated insulated beakers covered with a glass lid. Tasters were instructed to briefly lift the lid and sniff the headspace. For oral perception, beakers were replaced with preheated cups. The panel included 16 tasters, and each test was duplicated.

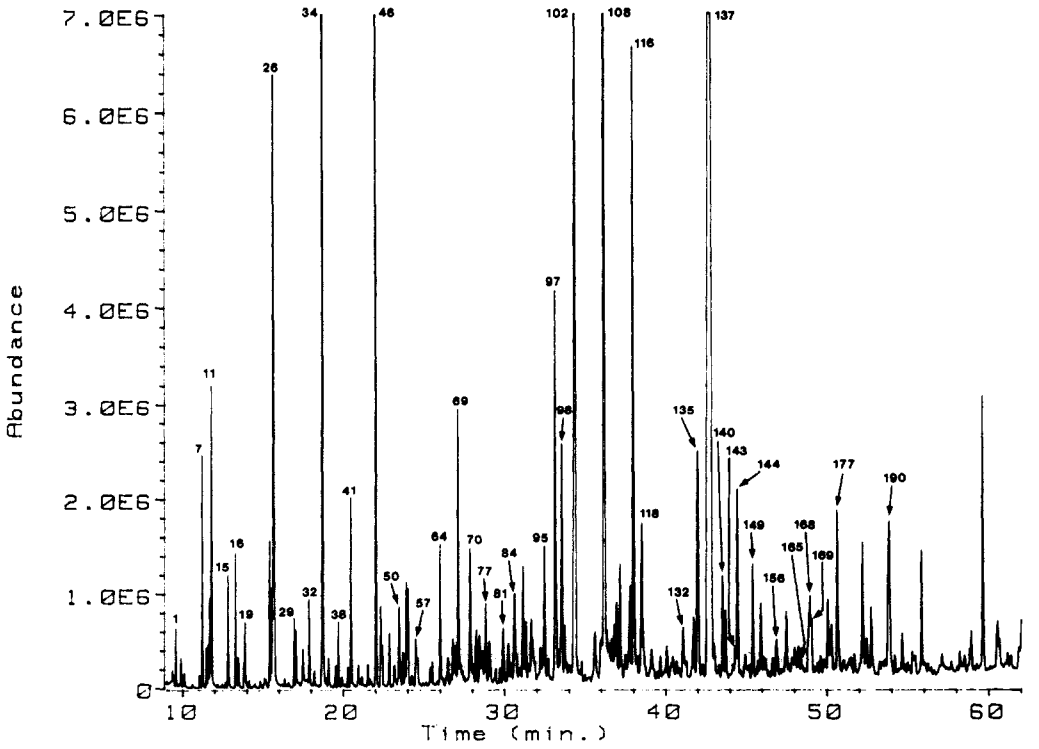

Figure 3. Chromatogram of Puerto Rico A coffee extract analyzed on a 50 m × 0.3 mm HP-5 fused-silica capillary column. Peak identifications are reported in Table III.

Table III. Components Identified in Puerto Rico A (Rio Coffee) and Santos Control Green Coffee Extracts[a]

peak	compound	ret index (Kovats) sample	ret index (Kovats) ref	Rio	control
1	ethyl acetate	551	595	+	+
7	3-methylbutanal	632	625	+	+
8	benzene	640	na	+	+
10	2-methylbutanal	645	635	+	+
11	butanol	647	655	+	+
15	2-pentanone	679	672	+	+
16	pentanal	693	694	+	+
18	1,4-dioxane (T)	705	na	+	-
19	3-hydroxy-2-butanone	706	na	+	+
24	3-methylbutanol	734	na	+	+
25	2-methylbutanol	738	na	+	+
26	pyridine	739	712	-	+
29	toluene	764	na	+	+
30	pentanol	766	756	+	+
32	2-pentenal (T)	782	na	+	+
34	hexanal	798	780	+	+
37	butyl acetate	814	793	+	-
38	butyl iodide (T)	816	na	+	-
41	furfural	841	815	+	+
46	ethylbenzene (T)	868	na	+	-
47	hexanol	868	858	+	-
48	dimethylbenzene (T)	868	na	+	-
51	styrene	893	874	+	-
54	2-heptanol	898	888	+	-
57	2,5-dimethylpyrazine	911	893	+	+
67	2-heptenal	957	940	+	+
69	benzaldehyde	962	947	+	+
76	trimethylbenzene (T)	995	na	+	-
77	decane	998	1000	+	-
84	benzyl alcohol	1037	1033	+	-
87	phenylacetaldehyde	1048	1024	+	+
90	2-octenal	1059	1045	+	-
95	linalool oxide	1077	1068	+	-
96	2-ethyl-3,6-dimethylpyrazine (T)	1082	na	+	-
97	butyl 3-methylbutanoate (T)	1093	na	+	-
98	linalool	1101	1092	+	+
99	nonanal	1105	1087	+	+
102	2-phenylethanol	1121	1104	+	+
108	2-nonenal	1163	1146	+	+
112	linalool oxide	1180	1164	+	-
113	2-methoxy-3-isobutylpyrazine	1185	1161	+	+
115	α-terpineol	1199	1185	+	-
116	methyl salicylate	1204	1181	+	+
132	ethyl salicylate	1280	1257	+	-
134	2,4-decadienal	1298	1288	+	+
135	indole	1303	na	+	+
136	methylnaphthalene	1310	na	+	+
137	4-vinylguaiacol	1328	na	+	+
140	2,4,6-trichloroanisole	1345	na	+	-
143	2,4,6-trichlorophenol	1365	na	+	-
144	γ-nonalactone	1372	1328	+	+
149	β-damascenone	1397	1371	+	+
151	vanillin	1412	1392	+	+
155	geosmin	1423	na	+	-
156	dichlorodimethoxybenzene (T)	1439	na	+	-
165	methyltrichloroanisole (T) + unknown	1483	na	+	-
169	phenethyl ester (T)	1502	na	+	-

[a] Same peak numbering as in Figure 3. T = tentative identification. na = not available.

RESULTS AND DISCUSSION

Preliminary Investigations. The results of a first investigation of Rio coffee flavor have been reported elsewhere (Spadone and Liardon, 1988). At that stage, the volatiles isolated by simultaneous distillation–extraction from a sample of green coffee (Puerto Rico A) possessing a pronounced Rio off-flavor were analyzed and compared with the extract of a reference coffee (Santos control). The examination of the chromatographic profiles revealed some quantitative differences, e.g., 4-vinylguaiacol was slightly more abundant in the Rio extract, but we could not single out any component specifically present in the Rio samples.

On the other hand, the use of the GC–sniffing technique permitted the location in the chromatogram of the Rio coffee extract of two peaks characterized by musty and earthy odors, reminiscent of Rio off-flavor. These odors were not detected when the reference coffee extract was analyzed, despite the presence of peaks at the same retention times. This is illustrated in Figure 1. Reinvestigating this portion of the two chromatograms by GC–MS led to the identification of 2,4,6-trichloroanisole and geosmin (Table I), two known very potent odorous compounds (Murray et al., 1975; Gerber, 1979; Maarse et al., 1985). The mass spectral and GC retention data obtained with authentic samples of these two compounds confirmed their identification.

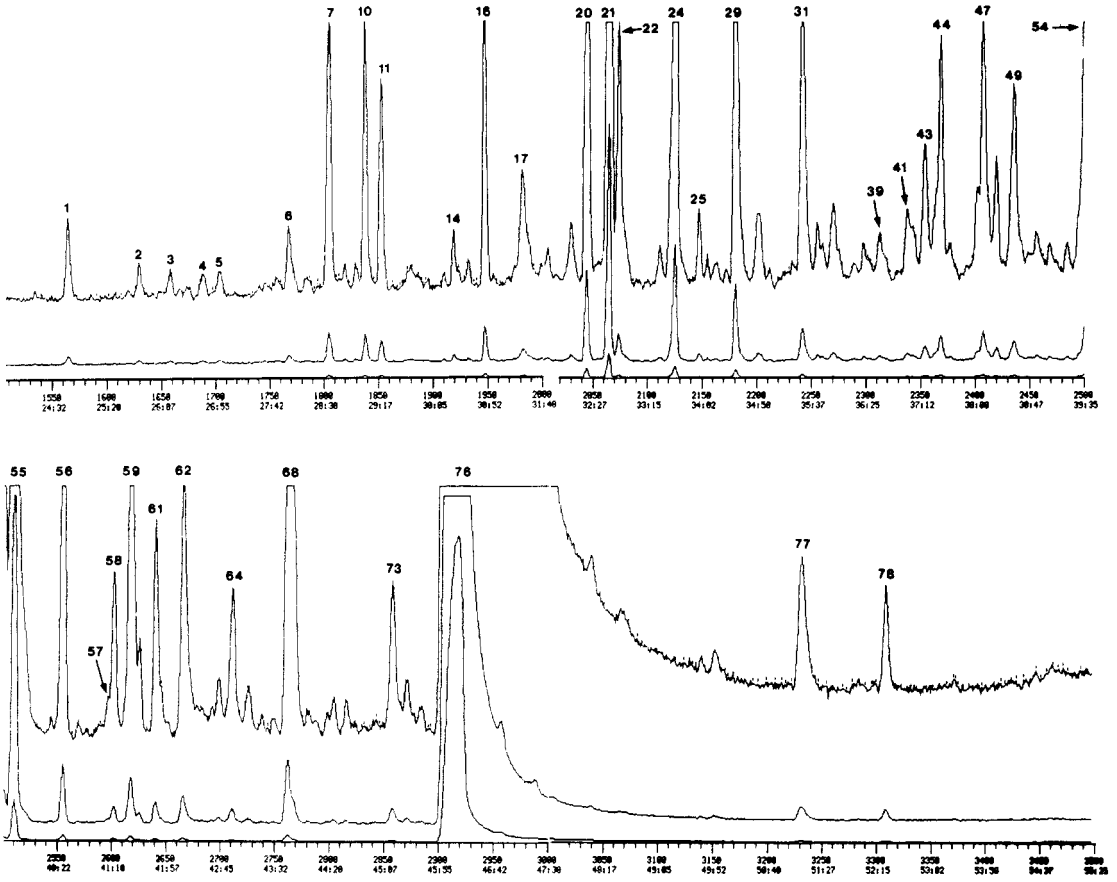

Figure 4. Chromatogram of cut 1 fraction on a 50 m × 0.3 mm Carbowax 20M fused-silica capillary column. Peak identifications are reported in Table VI.

Following this observation, eight other Rio coffees (listed in Table V) were analyzed for the possible presence of TCA and geosmin. TCA was found in all of these samples, while geosmin appeared to be unique to Puerto Rico A sample. (The presence of geosmin in this sample might be related to the observation that this coffee presented other defects, beside Rio off-flavor).

From these results, it appeared that TCA might play a major role in Rio flavor. However, several points remained open: (i) There was still the possibility of other components contributing to the off-flavor. (ii) Quantitative data were needed to characterize the significance of TCA in Rio coffee. (iii) More information was necessary to understand the origin of TCA in Rio coffee.

Detailed Analytical Investigation. In the second part of this study, we applied a systematic approach, including parallel flame ionization and odor detection, bidimensional gas chromatography, and GC–MS. This investigation was performed on Puerto Rico A green coffee extract, being the most abundant Rio coffee available and presenting the highest TCA content.

This was first analyzed by gas chromatography using a 30 m × 0.32 mm (i.d.) DB-5 fused-silica capillary column equipped with an effluent splitter with half of the sample going to an FID and the other half to a sniffing port. The chromatogram corresponding to the FID response is shown in Figure 2. Column effluent sniffing was performed on a series of dilutions of the concentrated extract, with the same approach as described by Acree et al. (1984) and Ullrich and Grosch (1987). At 243-fold dilution, 20 peaks still gave a perceptible odor. These peaks are listed in Table II, along with the perceived odor quality, retention indices, and partial identification based on GC–MS data.

These results seemed to confirm that TCA and geosmin were important contributors to the sample off-odor. However, it was evident from the mass spectral data that there were many overlapping peaks in the regions where the musty/earthy off-odors were detected. Certain odors might also have escaped olfactory detection due to the masking effect of closely eluting odorants (e.g., in lower dilution samples, TCA odor was masked by the closely eluting 4-vinylguaiacol). For these reasons, attempts were made to achieve a higher resolution of the coffee constituents.

To this effect, the extracts of Puerto Rico A and of the reference coffee were analyzed by GC–MS using a 50 m × 0.32 mm (i.d.) HP-5 fused-silica capillary column. The resulting chromatogram is shown in Figure 3. As expected, a better separation was achieved on the longer column. A list of the components identified is reported in Table III. For most of these compounds, the identification was achieved from the combined mass spectral and GC retention data. From these results, it appeared that the Rio coffee sample contained not only TCA but also other chlorinated aromatic compounds. Another interesting observation was the identification of butyl iodide in the Rio sample. On the other hand, various known odorous compounds, like 4-vinylguaiacol, methoxyisobutylpyrazine, β-damascenone, or vanillin, were detected in both samples in similar amounts and therefore could not be related to the Rio off-odor.

Another approach to resolve the components eluting in the region the chromatogram where musty and earthy odors had been detected (Figure 2) consisted of collecting this fraction (cut 1) in short sections of uncoated fused-silica capillary and reinjecting it into a DB-WAX capillary column. The resulting chromatogram is displayed

Table IV. Composition of Cut 1

peak	compound
1	γ-lactone
2	2-tridecanone
7	6,10-dimethyl-2-undecanone
10	MW 192
11	MW 222
16	sesquiterpene
20	2,4,6-trichloroanisole
20b	2,4-decadienal
21	β-damascenone
22	geosmin
22b	MW 150
24	6,10-dimethyl-5,9-undecadien-2-one
25	butyl benzoate
29	1,2-dimethoxy-4-ethylbenzene
30	benzyl 3-methylbutanoate
31	quinoxaline
32	terpenoid
34	MW 166
39b	methyltrichloroanisole
41	MW 156
42	phenylacetic acid
43	dimethylnaphthalene
44a	methyl 2-hydroxy-3-methylbutanoate
44	2-phenylethyl ester
46	MW 164
47	2-phenylethyl ester
47b	1,1-biphenyl
48	MW 172
49a	dimethylnaphthalene
49	MW 208
54	1-(4-hydroxy-3-methylphenyl)propan-2-one
55	γ-nonalactone
56	dichlorodimethoxybenzene
57	dichlorodimethoxybenzene
60	trimethoxybenzene
61	methyl 2-ethyl-6-hydroxybenzoate
64	5-methoxy-6,7-dimethylbenzofuran (T)[a]
65	methylquinoline
68b	γ-decalactone
76	4-vinylguaiacol
77	2,4,6-trichlorophenol
78	phthalic acid ester

[a] T = tentative identification.

Table V. Chlorophenol and Chloroanisole Concentrations in Rio Coffees

sample designation[a]	2,4,6-TCP, ppb	2,4,6-TCA, ppb
Puerto Rico A	33	106
roasted		61
Puerto Rico B	7	45
Sul de Minas	42	78
Brazil D	10	15
Caratinga F	3	1
Caratinga G	4	1
Santos Rio		53
roasted		24
Brazil C		12
Brazil E		37
Santos control	nd	nd

[a] All coffees green unless otherwise stated. nd = not detected.

in Figure 4, and the components identified are listed in Table IV. These results confirmed the presence in the Rio coffee of the chlorinated aromatics already listed in Table III but revealed the existence of two isomers of dichlorodimethoxybenzene. The odor contributions of the components in cut 1 were also evaluated by GC–sniffing. Again, none of the detected odors presented any obnoxious character, except for the musty and earthy odors perceived at TCA and geosmin retention times. TCP, which exhibits an odor similar to that of TCA but with a much higher perception threshold (Maarse et al., 1987), was not detected by sniffing.

Table VI. Relationship between TCA Concentration and Odor Detection by GC–Sniffing

TCA injection amt, fg	% positive detection		
	assessor 1	assessor 2	assessor 3
500	100	100	100
100	100	100	67
50	100	67	0
25	33	0	0
10	0	0	0

Table VII. TCA Sensory Detection Threshold in Coffee and Other Beverages

beverage	threshold value, ng/L	reference
coffee[a,b]	8	this work
coffee[a,c]	1–2	this work
water[b]	$(3–8) \times 10^{-2}$	Curtis et al. (1972)
		Maarse et al. (1987)
beer[b]	7	Maarse et al. (1987)
wine[b]	10	Tanner et al. (1981)

[a] Coffee infusions prepared at 50 g/L dosage. [b] Odor threshold value. [c] Flavor threshold value.

Analysis of Polychlorinated Phenols and Anisoles. Considering that, in most off-flavor cases involving TCA, other tri-, tetra-, and pentachlorophenols and -anisoles had also been detected in the tainted products (Maarse et al., 1987), a specific qualitative and quantitative search for this class of compounds was performed on six Rio green coffee samples. (At that time, the three other Rio samples were no longer available.) A sensitive method was developed to this effect based on selected ion monitoring mass spectrometry.

The results of these determinations are reported in Table V. As can be seen, the presence of TCP and TCA was confirmed in the six Rio coffees (TCA being also detected in the three other samples), in concentrations ranging from 1 to 100 ppb (ng/g). On the other hand, no other polychlorophenol or -anisole could be detected.

The influence of roasting on TCA concentration was also investigated on two Rio coffees (Puerto Rico A and Santos Rio). The data in Table V show that roasting only reduced TCA content in the beans to about 50%.

TCA Sensory Significance. The sensory significance of TCA was evaluated by measuring two different parameters: (i) the lowest detectable amount by GC–sniffing and (ii) the perception threshold in the coffee brew. The results of the first test are reported in Table VI. Taking into account that only half of the injected amount reached the sniffing port, these results indicate an LDA (50% positive perception level) of ca. 25 fg for TCA. Under the experimental conditions (makeup air flow rate 50 mL/min; TCA peak width 5–8 s), this was equivalent to a concentration in air of ca. 4 fg/mL, the same value as reported by Maarse et al. (1987).

TCA sensory threshold values in coffee, as determined by a tasting panel, are reported in Table VII where they are compared with values from the literature. As can be seen, TCA odor threshold in coffee is much higher than in water, probably resulting from a reduced volatility due to a coffee matrix effect and from a masking of TCA odor by coffee aroma. On the other hand, TCA odor threshold values in coffee and beer are almost identical. Interestingly, a lower threshold was found for oral perception than for direct olfaction. These thresholds,

Table VIII. Description of TCA Off-Note in Coffee by Tasting Panel

Direct Olfaction
dusty, *rioy*, *musty*, earthy, woody, wine cork taint, cereal, stale, *iodine*, *phenolic*
Oral/Retronasal Perception
bitter, burned, rubbery, *rioy*, *phenolic*, acrid, pungent, earthy, wine cork taint, *musty*, stale

determined for coffee brew, were also expressed as concentration in roast coffee, assuming a complete extraction during brewing. The resulting values were 0.16 ppb for the odor threshold and 0.02 ppb for the taste threshold. A comparison of these values with the data in Table V shows that in all Rio coffees investigated the content of TCA was significantly above the perception threshold, sometimes by several orders of magnitude.

The tasters, who for the most part were not familiar with Rio coffee characteristics, were also asked to describe the off-flavor perceived. The reported descriptors are listed in Table VIII. As can be seen, the characters traditionally associated to Rio flavor (phenolic, iodine-like) are included in the list, as well as the musty note perceived by us for the Rio coffee samples. The reference made by some tasters to "wine cork taint" was an indirect confirmation of the findings of Buser et al. (1982).

A final confirmation of TCA key role in Rio flavor was obtained from coffee experts at Illycaffè (Trieste, Italy) who positively recognized the Rio flavor in control coffee samples spiked with ca. 25 ppb TCA (E. Illy, private communication).

CONCLUSION

The analytical and sensory data reported here clearly indicate that TCA is the key compound in Rio coffee off-flavor. This compound was found in all Rio coffee samples investigated, with concentrations several orders of magnitude above its odor and taste thresholds in coffee brew. It was also established that TCA is only partly eliminated during coffee roasting. On the other hand, geosmin, found in only one of the Rio samples, is not related to this particular defect.

Off-flavor due to the occurrence of TCA is not a new problem. This compound has already been reported in a number of different food products: eggs and broilers (Engel et al., 1966; Curtis et al., 1972; Bemelmans and ten Noever de Brauw, 1974), chilled meat (Whitfield et al., 1987), packaged rice and flour, dried fruits (Whitfield et al., 1985), cocoa powder (Whitfield et al., 1984), wine (Tanner et al., 1981; Buser et al., 1982), brandy (Maarse et al., 1985), etc. An extensive review of TCA occurrence and formation pathways was presented recently by Maarse et al. (1987).

In most cases reported so far, the occurrence of TCA appeared to be related to some form of industrial contamination, e.g., water chlorination and use of chlorinated pesticides or fungicides. These primary contaminants would undergo various chemical or microbial degradation processes, leading, among others, to TCP (Ide et al., 1972; Engst et al., 1977; Maarse et al., 1987), which would then be converted into TCA. A number of mold strains have been found capable of performing this conversion (Curtis et al., 1974; Gee and Peel, 1974).

In Rio coffee, TCP is probably also the direct precursor of TCA. This assumption is supported by the simultaneous occurrence of the two compounds in all samples investigated and by the fact that molds isolated from Rio coffee beans have been found capable of converting chlorophenols into the corresponding chloroanisoles (Spadone et al., 1987). On the other hand, the origin of TCP is not clear. Although traces (2–30 ppb) of lindane have recently been found in coffee cherries (Cetinkaya et al., 1985), the absence of any other chlorophenols seems to exclude the possibility that this compound or any other industrial contaminant be the precursor of TCP.

Alternatively, TCP might be a natural metabolite of the molds infesting Rio beans. Indeed, a number of fungi are known to be capable of producing chlorinated metabolites (Strunz, 1973; Neidleman and Geigert, 1986), and some of the reported metabolites are structurally close to the various chlorinated compounds identified in this study (Singh and Rangaswami, 1966; Butruille and Dominguez, 1972). It is also interesting to note that TCA was already proposed to be of natural origin in several essential oils, where this substance was the only chlorinated compound detected (Stoffelsma and de Roos, 1973).

The hypothesis of the natural occurrence of TCP in Rio coffee is currently under investigations.

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Registry No. 2,4,6-TCA, 87-40-1; 2,4,6-TCP, 88-06-2; geosmin, 19700-21-1; ethyl acetate, 141-78-6; 3-methylbutanal, 590-86-3; benzene, 71-43-2; 2-methylbutanal, 96-17-3; butanol, 35296-72-1; 2-pentanone, 107-87-9; pentanal, 110-62-3; 1,4-dioxane, 123-91-1; 3-hydroxy-2-butanone, 513-86-0; 3-methylbutanol, 123-51-3; 2-methylbutanol, 137-32-6; pyridine, 110-86-1; toluene, 108-88-3; pentanol, 30899-19-5; 2-pentenal, 764-39-6; hexanal, 66-25-1; butyl acetate, 123-86-4; butyl iodide, 542-69-8; furfural, 98-01-1; ethylbenzene, 100-41-4; hexanol, 25917-35-5; dimethylbenzene, 1330-20-7; styrene, 100-42-5; 2-heptanol, 543-49-7; 2,5-dimethylpyrazine, 123-32-0; 2-heptenal, 2463-63-0; benzaldehyde, 100-52-7; trimethylbenzene, 25551-13-7; decane, 124-18-5; benzyl alcohol, 100-51-6; phenylacetaldehyde, 122-78-1; 2-octenal, 2363-89-5; linalool oxide, 1365-19-1; 2-ethyl-3,6-dimethylpyrazine, 13360-65-1; butyl 3-methylbutanoate, 109-19-3; linalool, 78-70-6; nonanal, 124-19-6; 2-phenylethanol, 60-12-8; 2-nonenal, 2463-53-8; 2-methoxy-3-isobutylpyrazine, 24683-00-9; α-terpineol, 98-55-5; methyl salicylate, 119-36-8; ethyl salicylate, 118-61-6; 2,4-decadienal, 2363-88-4; indole, 120-72-9; methylnaphthalene, 1321-94-4; 4-vinylguaiacol, 7786-61-0; γ-nonalactone, 104-61-0; β-damascenone, 23726-93-4; vanillin, 121-33-5; dichlorodimethoxybenzene, 72361-17-2; methyltrichloroanisole, 53452-80-5; 2-tridecanone, 593-08-8; 6,10-dimethyl-2-undecanone, 1604-34-8; 6,10-dimethyl-5,9-undecadien-2-one, 689-67-8; butyl benzoate, 136-60-7; 1,2-dimethoxy-4-ethylbenzene, 5888-51-7; benzyl 3-methylbutanoate, 103-38-8; quinoxaline, 91-19-0; phenylacetic acid, 103-82-2; dimethylnaphthalene, 28804-88-8; methyl 2-hydroxy-3-methylbutanoate, 34293-67-9; 1,1'-biphenyl, 92-52-4; 1-(4-hydroxy-3-methylphenyl)propan-2-one, 88659-81-8; trimethoxybenzene, 63744-60-5; methyl 2-ethyl-6-hydroxybenzoate, 55836-64-1; 5-methoxy-6,7-dimethylbenzofuran, 35355-35-2; methylquinoline, 27601-00-9; γ-decalactone, 706-14-9.

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