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Chemical Composition of Vintage Preban Absinthe with Special Reference to Thujone, Fenchone, Pinocamphone, Methanol, Copper, and Antimony Concentrations

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Thirteen samples of authentic absinthe dating from the preban era (i.e., prior to 1915) were analyzed for parameters that were hypothesized as contributing to the toxicity of the spirit, including naturally occurring herbal essences (thujone, pinocamphone, fenchone), methanol, higher alcohols, copper, and antimony. The total thujone content of preban absinthe was found to range between 0.5 and 48.3 mg/L, with an average concentration of 25.4 ± 20.3 mg/L and a median concentration of 33.3 mg/L. The authors conclude that the thujone concentration of preban absinthe was generally overestimated in the past. The analysis of postban (1915–1988) and modern commercial absinthes (2003–2006) showed that the encompassed thujone ranges of all absinthes are quite similar, disproving the supposition that a fundamental difference exists between preban and modern absinthes manufactured according to historical recipes. Analyses of pinocamphone, fenchone, base spirits, copper, and antimony were inconspicuous. All things considered, nothing besides ethanol was found in the absinthes that was able to explain the syndrome "absinthism".

KEYWORDS: Absinthe; thujone; fenchone; pinocamphone; Artemisia absinthium L.; wormwood; copper; antimony; higher alcohols

INTRODUCTION

The composition, and in particular the thujone content, of absinthe manufactured in the so-called "preban" era (the period of time that ranges from ca. 1805 until the banning of absinthe in France, in 1915) is a matter of debate, a debate that has been bedeviled by the uncritical repetition of figures derived from theoretical calculations from secondary sources, not actual chemical analysis of samples from the era (1-4).

Thujone is a natural essence typically associated with common wormwood (*Artemisia absinthium* L.) and Roman wormwood (*Artemisia pontica* L.), which are together two of absinthe's most widely used ingredients, besides green anise (*Pimpinella anisum* L.), hyssop (*Hyssopus officinalis* L.), lemon balm (*Melissa officinalis* L.), and Florence fennel (*Foeniculum vulgare* Mill.). Thujone (β -thujone in particular) is an epileptiform convulsant and is widely regarded as the "active" ingredient in absinthe (5, 6). It is certainly at the root of absinthe's reputation as being more drug than drink. In evaluating the validity of the extensive 19th and early 20th century scientific literature on the deleterious effects of absinthe, and its possible relevance to the public health issues related to modern absinthe (manufactured after its legalization in the European Union (EU) in 1988), a critical variable is the thujone concentration of preban or vintage absinthe. In other words, it remains to be determined if modern absinthes made from published 19th century recipes are chemically similar to those actually made by the large commercial manufacturers of the preban era. A thorough review of the extensive peer-reviewed literature shows, extraordinarily, only one actual test of a preban absinthe sample by Hutton (7). Other authors generally use assumed figures based on theoretical calculations or the analysis of samples from the 'postban' era (i.e., the decades that followed the 1915 ban on absinthe production in France). Of particularly profound influence was a paper by Strang et al. (8), which claimed a figure of 260 mg/L as being representative of the thujone concentration in preban absinthe. Although this figure was not based, by the author's own admission, upon actual chemical analyses (and in fact the theoretical basis on which it was derived is not completely clear), it has been uncritically quoted dozens of times since and has become for many authors the "conventional wisdom". Most recently for example, Huisman

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et al. make the claim in their paper "Absinthe—Is its history relevant for current public health?" that historic absinthe contained much higher concentrations of thujone than modern commercial offerings (9).

The problem of the lack of chemical analysis has been compounded by estimations of theoretical thujone content derived from clearly unreliable recipes, from the misinterpreted application of such recipes, or from the overestimation of the initial thujone content of the wormwood plant. The authors dealt with these theoretical aspects in a prior study, specifically by conducting a literature review on the composition of wormwood (A. absinthium and A. pontica), and found that the levels of thujone in the plant were overestimated in previously accepted calculations, which were liberally applied in estimating the thujone content of absinthe (3). Taking into consideration the incomplete extraction of wormwood during the maceration and distillation stages of absinthe production, the authors calculated that for typical French and Swiss 19th century recipes, the thujone content of absinthe may have ranged around a mean of 17-23 mg/L with large standard deviations of 16-21 mg/L, while the median content may have been around 10-14 mg/ L. The minimum possible concentration was 0 mg/L (if thujonefree wormwood chemotypes were used) up to a maximum of 58-76 mg/L (if oil-rich varieties with high thujone concentrations were utilized). Recently, the authors' calculations were confirmed by Rychlak, who calculated average thujone concentrations between 8.5 and 11.1 mg/L for preban absinthes using different recipes (10).

The current study was conducted with the intention of experimentally proving or disproving those theoretical calculations by analyzing an authentic collective of preban absinthes, the first time such a wide ranging analysis of absinthe from the preban era has been attempted.

In addition, the samples were analyzed for other parameters that were hypothesized as contributing to the toxicity of preban absinthe, including both naturally occurring herbal essences (e.g., pinocamphone, fenchone), methanol, higher alcohols, copper, and antimony.

MATERIALS AND METHODS

Samples. Beginning over 3 years ago in December 2004, an extensive effort has been undertaken by the authors to obtain as many samples of preban absinthe as possible, as well as a range of samples from the so-called "postban" period (i.e., absinthes made after 1915 in countries such as Spain, where the drink was never banned). The bottles from which the samples have been drawn were found in France, Switzerland, Spain, Italy, The Netherlands, and the United States. Only bottles meeting the following standards were used.

- (1) All bottles were full size (1000 mL) or half-size (500 mL). No 50 mL mini or "mignonette" bottles were used, primarily due to the difficulty in validating an original seal with these small bottles.
- (2) All bottles retained the seal of the original manufacturer, usually sealed in wax on top of the cork. Some bottles had a stamped tin seal instead of wax.
- (3) When the corks were removed, in all cases the cork was consistent with the age of the bottle.
- (4) The bottles gave every indication of authenticity, with absolutely no signs of tampering or alteration. They also gave every indication of having been adequately stored, bearing no evidence of excessive exposure to light or heat.
- (5) The provenance of all bottles is known. They all come from primary sources (i.e., original cellars dating back to the early 1900s).
- (6) The bottles all had their original, intact labels.
- (7) The bottles all exhibited nearly original content levels, in most cases around 90% full. This level of ullage is consistent with a century-old bottled spirit and is an indication generally of good

storage and, more specifically, that the cork has continued to provide a relatively airtight seal over the life of the bottle.

- (8) The contents of the bottles were in all cases organoleptically consistent with vintage absinthe.
- (9) The preban brands sampled were all among the best selling and highest quality of the era. All were, on the evidence of the manufacturer's own marketing materials, made by distillation from whole herbs, without the use of essences or artificial additives. Incidentally, the vast majority of surviving bottles from the preban era identified to date are those that represent the best selling, highest quality brands.

In some cases, the bottles were opened conventionally, by removal of the cork with a corkscrew. Great care was taken not to allow any contaminants from the cork itself or the wax seal to enter the absinthe during this process. In the majority of cases, however, the samples were extracted by use of a spinal stent (a hypodermic needle with an inner pin) pushed through the seal and the cork, and a syringe to draw out the liquid (see Supporting Information). The samples (approximately 30 mL) were filled in standard laboratory glass vials with screw cap and sent by courier mail to the CVUA Karlsruhe for analysis, which was started immediately upon arrival of the samples.

The modern commercial absinthes were sampled in the context of official food control by governmental food inspectors in the German Federal State Baden-Württemberg. The authors only selected modern samples that were judged to be authentic absinthes according to refs *11*, *12*. In addition, the authors included a sample of a Swiss product bought in Switzerland after the repeal of the ban in that country, which was enacted in 2005.

Reagents and Materials. All reagents consisted of analytical grade standards. An α , β -thujone isomer mixture, α -thujone, and cyclodecanone were procured from Fluka (Buchs, Switzerland). Fenchone, methanol, acetaldehyde, 1-propanol, 1-butanol, 2-butanol, isobutyl alcohol, 2-/3-methyl-1-butanol, 2-phenylethanol, 1-hexanol, benzyl alcohol, methyl acetate, ethyl acetate, benzyl acetate, ethyl lactate, ethyl caprylate, ethyl benzoate, and benzaldehyde were obtained from Sigma-Aldrich (Taufkirchen, Germany). Pinocamphone (3-pinanone) was synthesized according to Höld et al. (13). The authors were unable to confirm the conformation of pinocamphone; therefore, the sum of *cis*and *trans*-3-pinanone is reported.

Gas Chromatography and Mass Spectrometry for Thujone, Pinocamphone, and Fenchone. A previously described procedure was used (14), which was extended for the analysis of pinocamphone and fenchone. The sample preparation was accomplished using liquid–liquid extraction with 1,1,2-trichloro-1,2,2-trifluoroethane after the method of Rapp et al. (15). After addition of 350 μ L of cyclodecanone as internal standard (freshly prepared methanolic solution, 20 μ g/mL), 2 mL of sample was shaken with 10 mL of ethanol (15% v/v) and 1 mL of 1,1,2-trichloro-1,2,2-trifluoroethane for 60 s, and the sample was centrifuged at 3000 rpm for 5 min for phase separation. The lower organic phase was drawn off using a transferpettor and filled a GC vial. For calibration, thujone, fenchone, and pinocamphone solutions (0.1–40 mg/L) were freshly prepared in ethanol (15% v/v) and extracted as the samples. The organic extracts were stored at 4 °C until analysis.

The GC–MS system used for analysis was an Agilent model 6890 Series Plus gas chromatograph in combination with a CTC Combi PAL autosampler and an Agilent 5973N mass selective detector. Data acquisition and analysis were performed using standard software supplied by the manufacturer. Substances were separated on a fused silica capillary column (HP-Innowax, 60 m × 0.25 mm i.d., film thickness 0.25 μ m). The temperature program was 45 °C hold for 1 min, 5 °C/min up to 180 °C, 25 °C/min up to 240 °C, hold for 5 min. The temperatures for the injection port, ion source, quadrupole, and interface were set at 240, 230, 150, and 250 °C, respectively. Split/ splitless injection mode (1 μ L, splitless) and helium with a flow rate of 1.0 mL/min as carrier gas were used.

To determine the retention times and characteristic mass fragments, electron impact (EI) mass spectra of the analytes were recorded by total ion monitoring. The analytes α -thujone, β -thujone, fenchone, *cis*-3-pinanone, and *trans*-3-pinanone as well as the internal standard cyclodecanone were baseline separated. The retention times were 14.2 min for fenchone, 17.2 and 18.0 min for the 3-pinanone isomers, 14.9

Table 1. Analysis Results of Preban, Postban, and Modern Legal Absinthes for Thujone, Pinocamphone, and Fenchone^a

sample		α -thujone	β -thujone	total thujone	fenchone	total pinocamphone						
no.	sample name	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)						
Preban Products												
P1	Bazinet, France, ca. 1910	5.1	32.0	37.1	6.7	0.6						
P2	Premier Fils, France, ca. 1910	5.7	14.8	20.5	9.4	4.7						
P3	Edouard Pernod, Switzerland, ca. 1914	4.8	28.5	33.3	10.6	2.8						
P4	Edouard Pernod, Switzerland, 1905-1914	7.7	39.3	47.0	45.8	7.8						
P5	Berger, Switzerland, ca. 1910	0.2	0.3	0.5	2.9	9.8						
P6	Mattei, Corsica, ca. 1910	0.8	3.7	4.5	2.9	0.4						
P7	Dechanet, France, ca. 1910	0.1	1.6	1.7	5.2	nd						
P8	Pernod Fils, France, 1895–1905	1.4	2.2	3.6	0.1	2.4						
P9	Pernod Fils, France, ca. 1910	6.8	41.5	48.3	31.5	3.1						
P10	Pernod Fils, France, 1895–1905	0.9	0.6	1.5	nd	2.5						
P11	Pernod Fils, France, 1895–1905	6.5	36.7	43.2	27.6	3.6						
P12	Pernod Fils, France, 1895–1905	7.1	35.1	42.2	31.4	2.8						
P13	Pernod Fils, France, 1895–1905	7.4	39.5	46.9	31.4	2.9						
R1	Pernod File Spain Tarragona ca 1030	0.5	1 2	1.8	2.5	0.5						
B2	Pernod File, Spain, Tarragona, Ca. 1950	0.5	0.4	1.0	2.5	0.5						
B3	Pernod SA (successor to Edouard Pernod)	0.0	2.8	3.1	2.0	0.0						
00	Snain Tarragona ca 1935	0.2	2.0	0.1	50.0	0.4						
R4	Albado Habanna Cuba ca 1935	1.3	8.8	10.1	6.1	21.9						
B5	Argenti Spain ca 1940–1945	nd	nd	nd	1 1	nd						
B6	Argenti, Spain, ca. 1940–1945 Argenti Spain, ca. 1970	0.4	2.2	2.6	77	nd						
B7	Clandestine Switzerland Val de-Travers ca 1953	2.8	2.2	2.0	5.4	0.3						
B8	Clandestine, Switzerland, Val-de-Travers, ca. 1955	2.0	75	8.0	1.4	1.6						
BQ	Clandestine, Switzerland, Val-de-Travers, 2004	0.3	1.0	17	53	13						
B10	Clandestine, Switzerland, Val-de-Travers, 2004	0.5	9.0	9.4	12.8	6						
DIO		0.4	5.0	0.4	12.0	0						
• ·	0	Modern Legal Ab	sinthes									
A1	Germany, 2003	1.4	29.4	30.8	3.3	nd						
A2	France, 2003	6.2	4.7	10.9	6.1	nd						
A3	Germany, 2003	1.6	31.7	33.3	8.2	nd						
A4	France, 2003	5.6	5.0	10.6	5.3	nd						
A5	Austria, 2003	2.2	28.9	31.1	0.1	nd						
A6	France, 2003	0.9	20.6	21.5	8.4	nd						
A7	France, 2003	3.2	25.6	28.8	nd	0.3						
A8	Austria, 2005	3.5	0.7	4.2	95.6	2.9						
A9	Switzerland, Val-de-Travers, 2006	2.6	68.6	71.2	18	7.7						

^a nd: not detectable.

min for α -thujone, 15.4 min for β -thujone, and 22.6 min for cyclodecanone. For quantitative analysis, the chosen diagnostic mass fragments were monitored in the selected ion monitoring (SIM) mode. Fenchone: m/z 152 as target ion and m/z 81, 69, and 109 as qualifier ions. *cis-/ trans*-3-Pinanone: m/z 152 as target ion and m/z 83, 69, and 95 as qualifier ions. α,β -Thujone: m/z 110 as target ion and m/z 81 and 152 as qualifier ions. Cyclodecanone: m/z 111 as target ion and m/z 98 and 154 as qualifier ions. For quantification, peak area ratios of the analytes to the internal standard were calculated as a function of the concentration of the substances. The limit of detection was 0.08 mg/L. Further method validation data were reported in ref 14.

Volatile Constituents, Copper, and Antimony. The samples were analyzed for selected volatile constituents, including methanol, acetal-dehyde, 1-propanol, 1-butanol, 2-butanol, isobutyl alcohol, 2-/3-methyl-1-butanol, and ethyl acetate according to the European Community reference methods for the analysis of spirits (*16*). The exact details of the method were previously described for the analysis of Mexican spirits (*17*). The limit of detection was 0.5 g/hL of pure alcohol (pa) for all volatile constituents. Copper was analyzed using atomic absorption spectroscopy according to the German reference method (limit of detection, 0.01 mg/L) (*18*). Antimony was analyzed using semiquantitative inductively coupled plasma mass spectrometry (ICP-MS) after evaporation of the sample and reconstitution in ultrapure water (limit of detection 0.001 mg/L).

RESULTS AND DISCUSSION

Thujone. The analytical results for thujone in preban, postban, and selected modern absinthes are shown in **Table 1**. The total thujone content of preban absinthe was found to range

between 0.5 and 48.3 mg/L, with an average concentration of 25.4 ± 20.3 mg/L and a median concentration of 33.3 mg/L.

This experimentally determined range is in very good accordance with the authors' calculated range of between 0 and 76 mg/L mentioned above. The average experimental concentrations do also excellently correspond to the average calculated concentrations of 23 ± 21 mg/L (3). The results are also in excellent agreement with the earliest information about the thujone content of absinthe in the scientific literature. Wilson (19) estimated in 1936 that absinthe made from essences contained 1.8–45 mg/L and absinthe made with wormwood contained 2–34 mg/L of thujone.

The distribution in the samples is compared to the calculated values in **Figure 1**. Analysis of variance (ANOVA) shows that the means of the four absinthe subgroups (theoretical preban, experimental preban, postban, modern commercial) are not significantly different (p = 0.105). It can be concluded that the authors' previous calculations were valid and that the thujone concentration of preban absinthe was generally overestimated in the past. The claims of high thujone concentrations up to 260 mg/L are now clearly disproven by the results of this study. The encompassed thujone range of preban and modern high-quality absinthes is also quite similar, disproving the supposition that a difference exists between both groups.

Only six of the preban samples, one of the postban, and one of the modern absinthes had thujone levels above the current *Codex Alimentarius/*EU limit of 35 mg/L (20, 21). Singular cases





Figure 1. Box chart of total thujone concentrations. Comparison between the theoretical calculation for "absinthe ordinaire" according to ref *3* and the analysis results for preban, postban, and modern legal absinthes.

of modern absinthes above the limit have been previously observed (1). However, such samples found to be in excess of the limit cannot be treated as harmful to health, because the limit was of course derived using safety factors. The German Federal Institute for Risk Assessment holds the view that, even if the legal limit of 35 mg/L is clearly exceeded, the consumer does not ingest health-threatening amounts of thujone (22).

In the preban samples, the highest concentration of 48.3 mg/L was found in a Pernod Fils absinthe. The consumption of as much as 1 L of such an absinthe would result in an intake of about 0.8 mg of thujone/kg of body weight (kg bw) for a 60 kg adult. Even this unrealistically high intake of alcohol produces thujone concentrations below the "no observed effects level" (NOEL) of 5 mg/kg bw (for a detailed toxicological review about thujone, see Padosch et al. (4)). The consumption of one glass (30 mL) of such an absinthe, containing approximately 1.3 mg of thujone, would result in an intake of approximately 0.02 mg of thujone/kg bw, which is 250 times lower than the NOEL.

Other preban Pernod Fils absinthes exhibited lesser concentrations of 1.5, 3.6, 42.2, 43.2, and 46.9 mg/L. Samples of the same brand, bottled in the postban period after licensing the production of Pernod Fils to a distillery in Tarragona (Spain), exhibited thujone levels of only 1.0 and 1.8 mg/L. The analytical results likewise reveal that absinthes crafted in the postban period (the decades immediately following the 1915 ban) generally exhibit thujone concentrations that range from below detectable concentrations to 38.3 mg/L, with an average concentration of 7.6 \pm 11.4 mg/L and a median concentration of 2.9 mg/L. One possible explanation may be the fact that indigenous Spanish wormwood chemotypes may be nearly thujone-free (23). Another possible explanation is that postban producers in Spain may have employed lesser amounts of A. absinthium as a precautionary measure given the controversy that surrounded this herb and its primary use (the distillation of absinthe) during that period.

It is notable that even for the same manufacturer, and more so between the different preban absinthe producers, a substantial variation in thujone concentration is evident. Given this fact, it seems illogical to claim that a definitive or fixed thujone content threshold should be demanded from an absinthe. This reasoning is supported by the fact that preban products of high acclaim, but with low thujone content (e.g., the 1910 Dechanet or 1910 Berger), displayed the same familiar and desirable organoleptic properties as equally highly regarded absinthes with higher thujone content (e.g., Pernod Fils). In short, these results imply that no reasonable connection can be drawn between thujone concentration and flavor or quality, and that thujone, as such, cannot be tasted (i.e., organoleptically isolated) in finished absinthe.

Also notable is the fact that variability in the thujone (and fenchone, etc.) content of different samples of the same brand, produced and bottled in the same facility, and from the same period has been observed. There are two possible explanations for this: (1) variability as a result of extrinsic influences during storage and aging and (2) variability at the time of original bottling (e.g., due to regional and seasonal variety, herb chemotype, drying conditions, or adjustment of recipes).

The authors have not found any evidentiary or investigative support for explanation 1, i.e., the proposition that thujone content changes in bottle, as a result of aging or other environmental factors. Fröhlich and Shibamoto studied the stability of thujone in detail (24). No change of the thujone concentrations was observed during storage experiments in 100% ethanol and 30% ethanol at either pH 2.5 or 6.5. Only at pH 11.5 did a very rapid epimerization of α -thujone to β -thujone take place, reaching an equilibrium of approximately 1:2 α -thujone: β -thujone. A comparison of the reaction rates at 20 °C both in sunlight and in the dark illustrated that the reaction was independent of the influence of light. At different temperatures (100, 20, and 0 °C), nearly no difference in reaction rate was observed. Additionally, the authors previously tested the storage stability of authentic absinthes (14). The thermal exposure of absinthes did not express any statistically significant influence on thujone concentrations. No significant change in either thujone isomers was evident in absinthes stored for 1 year at ambient temperature.

Therefore, it is the authors' belief that the most likely explanation is the second one. These absinthes were distilled from whole herbs, usually dried for a period of between 6 and 12 months prior to use. From the experience of modern commercial absinthe distillers, it is clear that there is considerable regional and seasonal variation in the organoleptic properties of plant material used for the distillation of absinthe. This being evident, it has been proposed that, over time, the stillmasters at the large preban absinthe houses would have periodically adjusted their recipes, so as to maintain a consistent organoleptic profile, a process that would inevitably mean increasing or decreasing the relative quantities of wormwood, fennel, anise, etc. used. It is also likely that over time, herbs would have been sourced from various suppliers, and as a result, variations would have inevitably occurred in the herb chemotypes used. As illustrated by the authors in a previous article (3), in the case of wormwood in particular, very large variations in thujone content (0-70%) are possible between different chemotypes. The same reasons apply for the variability in the modern absinthes. The atypical sample A9 with 71.2 mg/L of total thujone was most likely due to the maceration of inadequately dried wormwood of a high-thujone chemotype and a lack of chemical quality control by the small Swiss artisanal distillery.

Fenchone and Pinocamphone. This study reports for the very first time fenchone and pinocamphone concentrations as determined in both preban and selected modern absinthes produced at the time of this writing (Table 1).

Neither the *Codex Alimentarius* nor the EU legislation provide limits for fenchone and pinocamphone in food. Hyssop and fennel both are "generally recognized as safe" by the U.S. Food and Drug Administration (25). In contrast, limits are provided by the French national legislation (5 mg/L for fenchone and 20 mg/L for pinocamphone) (26). The rationale for the limits may have been the fear of the return of absinthism in France after the EU's deregulation of absinthe. Therefore, the quantity of absinthes three major ingredients (wormwood, hyssop, fennel) was indirectly regulated by their constituents, thujone, pinocamphone. and fenchone.

Another possible rationale for the French limits concerns reports that aim to determine the toxicity of the essential oils of the aforementioned three herbs. Wormwood, hyssop, and fennel oil were considered to be convulsant essential oils with epileptogenic components (27). Essences of hyssop have caused poisoning of humans and were found to possess a convulsant action of central origin in animals. The neurotoxicity of hyssop appeared to be related to the presence of two terpene ketones, pinocamphone and isopinocamphone, the former of which has powerful convulsant properties and is lethal at doses above 0.05 mL/kg (28). Thujone and pinocamphone induce the same type of electrocortical seizures associated with myoclonic activities (29). Pinocamphone (like thujone) acts as a γ -aminobutyric acid (GABA) receptor antagonist (5, 13). However, the toxicological observations and studies concerning hyssop are limited and remain less than adequate for developing sound judgment concerning the use of this important food flavoring and traditionally medicinal herb (13).

The ISO standard with regard to the oil of hyssop (*Hyssopus officinalis* L.) recommends levels between 5.5 and 17.5% for *trans*-3-pinanone (pinocamphone) and levels between 34.5 and 50% for *cis*-3-pinanone (isopinocamphone) (30). It remains unclear in the French legislation if only the *trans*-isomer is regulated. However, as both pinocamphone isomers exhibit similar potency at the GABA receptor (13), it appears reasonable to limit both isomers or the sum of them, as is the practice with thujone. In this study, the sum of both pinocamphone isomers exceeds the limit of 20 mg/L in none of the preban absinthe samples, in only one of the postban products (21.9 mg/L pinocamphone). and in none of the modern products.

Hyssop was usually used in quantities of 500-1000 g per 100 L of distilled absinthe. In the case of absinthe from essences, 6 g of hyssop essence were used per 100 L of absinthe (31). Under consideration of the maximum total pinocamphone content according to ISO 9841 of 67.5% and the assumption of 1% essential oil in the hyssop herb, a maximal possible pinocamphone concentration in the unfinished distillate of 35 mg/L (500 g of herb), 68 mg/L (1000 g of herb), or 41 mg/L (6 g of essence) can be calculated under the hypothetical assumption that no loss during maceration and distillation would occur. As in the case of thujone, such losses appear to occur as the pinocamphone concentrations in the actual absinthes are significantly lower than those simply calculated values. It can be concluded that the determined pinocamphone concentrations do not offer a novel explanation for the syndrome known as "absinthism". The boiling point of pinocamphone is approximately 10° higher than that of thujone (thujone, 201 °C; pinocamphone, 212-214 °C); therefore, pinocamphone should exhibit similar behavior to that of thujone when distilled under similar conditions, and a significant discrimination during the distillation will occur (32).

Another explanation for the relatively low pinocamphone concentrations in absinthe may be due to the different varieties and chemotypes of hyssop (33). The chemical composition of hyssop oil varies considerably, even between oils obtained from different samples of the same variety of hyssop (34). Volatile oil composition of hyssop varies also in dependence on growth

stage or climatic conditions (*35*). Especially *Hyssopus officinalis* L. var. decumbens (Jordan & Fourr.) Briq. is morphologically different from *Hyssopus officinalis* L. and is peculiar for its lack of pinocamphone (*34*).

The rationale for the lower French maximum limit of fenchone (5 mg/L) than for pinocamphone (20 mg/L) and thujone (35 mg/L) is difficult to understand or to assess from a toxicological standpoint. The existing toxicological data on fenchone is sparser than those for pinocamphone. However, the LD_{50} of fenchone in rats was reported to be 6160 mg/kg (*36*), which is significantly higher than the LD_{50} of thujone, which was reported to be in the range of 192–500 mg/kg (*4*).

The French fenchone limit of 5 mg/L is exceeded by 9 of the 13 preban absinthes, 6 of the 10 postban products, as well as 6 of 9 modern absinthes. The maximum fenchone concentration recorded in a preban absinthe was 45.8 mg/L in an Edouard Pernod sample. The highest fenchone concentration of all, 95.6 mg/L, was measured in a modern absinthe. Many historic absinthe recipes demand a larger amount of fennel (by weight) than that of wormwood or hyssop. One to 2 kg of fennel are usually demanded in French absinthe "ordinaire" or absinthe "fine", and Swiss recipes even require up to 5 kg of fennel per 100 L of absinthe (31). For absinthe from essences, 30 g of fennel essence is demanded for 100 L of absinthe. If the fennel contains 4% of essential oil, with 20% of that amount consisting of fenchone, the unfinished distillate would contain a fenchone concentration of 80 mg/L (1 kg of fennel), 160 mg/L (2 kg of fennel), 400 mg/L (5 kg of fennel), or 60 mg/L (30 g of fennel essence). The actual concentrations in the preban absinthes were, again, lower than those simple calculations, because fenchone, like thujone and pinocamphone, appears to be discriminated during distillation.

The very strict French fenchone limit restricts the legal sale in France of some absinthes manufactured according to historical principles. In this respect, it is a significantly greater limiting factor than the EU thujone limits (and arguably an even less defensible one). Outside of France though, in the rest of the European Union as well as in Switzerland, fenchone is not restricted, and such products are marketable.

Methanol and Higher Alcohols. Beside issues associated with the quality and consistency of industrially prepared essential oils, another theory for absinthism was a substandard quality of alcohol used for absinthe production, with specific emphasis regarding toxic concentrations of methanol or higher alcohols (fusel oils). It was assumed that heads and tailings, which were separated from the product fractions during the distillation by legal manufacturers, were purchased by illegal manufacturers and used as a main component for adulterated products. Even inedible mixtures of methanol and fusel alcohol allegedly could be made drinkable by masking through the bitter wormwood taste (*37*).

Surprisingly, the analytical results indicate the preban absinthes exhibited a very clean base spirit (**Table 2**). These absinthes obviously were manufactured using highly rectified alcohol ("neutral alcohol" or "ethyl alcohol of agricultural origin"). For example, the maximum methanol concentration allowed by the current European requirements for neutral alcohol is 30 g/hL pa (g/hL of pure alcohol) (*38*), which is slightly exceeded only in the two samples of Edouard Pernod (54 and 79 g/hL pa). It should be noted, however, that the general methanol limit for spirits is higher than that for neutral alcohol (e.g., 1000 g/hL pa for fruit spirits). It cannot be excluded that both Edouard Pernod absinthes were manufactured using wine spirit, which may explain the higher concentrations of methanol.

Table 2. Analysis Results of Preban, Postban, and Modern Legal Absinthes for Methanol, Higher Alcohols^a, and Copper^b

sample	methanol (g/bl_pa)	acetaldehyde	1-propanol	2-/3-methyl-1-butanol	isobutyl alcohol	ethyl acetate	copper (mg/L)					
<u>no. (ynic pa) (ynic pa)</u>												
	22	15	1	2	nu 1	21	0.48					
P2	12	17	2	I	1	11	0.09					
P3	54 70	20	0	C	3	32	0.30					
P4	79	0	nu	nd	nu	8						
P5	2	na	na	nd	nd	4	0.15					
P0	9	3	2	l nd	nd	2	0.29					
P7	na	na	na	nd	na	na	na					
P8	3	1	na	nd	na	na	na					
P9	21	nd	9.	nd	1	12	0.39					
P10	3	2	nd	nd	nd	nd	nd					
P11	18	8	8	nd	1	9	nd					
P12	20	9	8	1	nd	9	0.17					
P13	24	11	13	2	3	13	0.13					
Postban Products												
B1	13	nd	3	nd	nd	5	0.59					
B2	12	nd	3	nd	nd	4	0.74					
B3	31	nd	4	nd	nd	27	0.34					
B4	5	14	30	5	16	10	0.43					
B5	7	8	0	nd	nd	5	nd					
B6	19	nd	nd	nd	nd	nd	0.14					
B7	3143	nd	nd	nd	nd	5	nd					
B8	13	nd	nd	1	nd	7	nd					
B9	7	nd	nd	nd	nd	3	nd					
B10	9	nd	nd	nd	nd	2	nd					
			Mor	lern Legal Absinthes								
Δ1	5	17	nd	nd	nd	nd	nd					
42	1	nd	nd	nd	nd	3	nd					
43	7	nd	nd	nd	nd	nd	nd					
A3 A4	1	nd	nd	nd	nd	1	nd					
A5	1	nd	nd	nd	nd	1	nd					
A5 A6	nd	nd	nd	nd	nd	2	nd					
A0 A7	nu 6	nd	nu	nd	nu	∠ nd	nd					
A/ A0	0	nu	nu	nd	nu	10	0.06					
AO	3	nu	110	01	nu e	4	0.20 nd					
Ag	U	nu	2	21	U	4	nu					

^a The following substances were not detectable in all samples: 1-butanol, 2-butanol, 1-hexanol, benzyl alcohol, 2-phenylethanol, methyl acetate, benzyl acetate, ethyl lactate, ethyl caprylate, ethyl benzoate, and benzaldehyde. ^b nd: not detectable.

The only abnormality regarding methanol was recorded from a sample of absinthe that was clandestinely manufactured in 1953, in the Val-de-Travers (Switzerland), which exhibited 3143 g/hL pa of methanol. This equates to approximately 1.7% vol of methanol in the absinthe, which nears the maximum tolerable concentration of methanol of 2% vol (39). The cause for the high methanol concentration is most probably the use of denatured industrial alcohol that usually contained around 5% vol of methylene (raw methyl alcohol produced from the dry distillation of wood) (40). Today, the use of methylene as denaturing agent has been discontinued in favor of less toxic substances. The clandestine Val-de-Travers absinthes distilled in 2004 (just before the repeal of the ban) displayed a very clean base spirit. Also worthy of note is the fact that, after the repeal of the absinthe prohibition in Switzerland in 2005, formerly clandestine but now legally produced absinthes are now under regulatory control similar to products produced in the EU.

In all samples of absinthes from all eras, the concentrations of the other higher alcohols were also relatively low (i.e., lower than what is found in current brandy, rum, or whisky samples) and give no toxicological concern. In general, this study did not reveal so much as one sample of preban absinthe that would be in jeopardy as a result of the quality of its base spirit, even by contemporary standards. All preban samples fit modern regulatory guidelines with respect to quality of alcohol used to craft them. The quality of alcohol in the modern absinthes was likewise found not to be objectable. In this regard, the authors can confirm the results of Skopp et al. (41), who investigated the alcoholic congeners of 56 brands of modern absinthe and reported that these spirit drinks were predominantly made on the basis of ethyl alcohol of agricultural origin, according to the European regulations.

Copper and Antimony. Aside from hypotheses of absinthism being rooted in toxic quantities of terpenes and an inferior quality of base spirits, copper and antimony were discussed as a potential cause. Inorganic salts like copper sulfate or copper acetate were said to have been added as adulteration to enhance or act as a substitute for the light green color of chlorophyll (42). However, the copper content in the more highly regarded preban absinthes was inconspicuous (Table 2). Most of these products exhibited concentrations below the current European drinking water maximum for copper of 2 mg/L (43). Only the preban Edouard Pernod exceeded this limit with 6.59 mg/L of copper. However, levels as high as 5.3 mg/L of copper were measured in current sherry brandies (44) or levels up to 9.2 mg/L (45) or even 14.3 mg/L (46) of copper in Brazilian sugar cane spirits. Copper levels of the magnitude typical of preban absinthes can be attributed to the copper stills used for distillation and/or copper receiving vessels as the most prevalent potential source. The authors did not find any evidence that copper salts were used as coloring in the absinthes under investigation.

Another form of adulteration was allegedly the addition of antimony salts to absinthe, which would make absinthe turn milky when adding water, simulating the so-called "louche effect" (1). None of the samples in our study contained detectable antimony levels.

The Exemplary Character of Preban Pernod Fils Absinthe. Pernod Fils was, by some distance, the largest of all absinthe manufacturers of the preban era. The center of the French absinthe industry was in the Franche-Comte and specifically the town of Pontarlier in the Haut Doubs. A 1905 table listing all the Pontarlier absinthe distilleries, 25 in all, showed they operated collectively 151 stills and produced in total just over 70 000 hL of absinthe (47). Of this, Pernod Fils alone operated 60 stills and produced 40 000 hL, in other words, more than the other 24 manufacturers together. Aside from their dominance in the French market, Pernod Fils was also almost certainly the largest exporter of absinthe, distributing their product in the U.S., U.K., throughout Europe, and in all the French colonies in South America, Africa, and Australasia (48). So dominant was Pernod Fils that the company name became a generic word for absinthe; consumers would automatically order "un Pernod" rather than "une absinthe". To capitalize on this, several offshoots of the Pernod family set up rival distilleries, some of them-Edouard Pernod, Legler-Pernod, Gempp-Pernod-being substantial companies but many of them much smaller, undercutting Pernod's prices while capitalizing on the fact that their product could also be called un Pernod and thus be assured of at least a degree of commercial success.

Pernod Fils was not only the biggest manufacturer but widely regarded at the time as the best. It sold for a premium price: at wholesale level, 2 fr per liter, versus 1.75 fr for Berger, 1.65 fr for Edouard Pernod, and 1.55 fr for Premier Fils, all three in themselves premium marques (49).

Finally, unique among the large absinthe producers of the preban era, we know a substantial amount about the ingredients and methods Pernod Fils used to manufacture its absinthe. The two profusely illustrated booklets the company produced in 1896 and 1905 give copious details of the herbs used and an outline of the distilling process (48, 50). It is clear from this text that Pernod Fils' basic recipe and process was in line with the methods outlined in Duplais' *Traité de la Fabrication des Liqueurs et de la Distillation des Alcools*, which went through seven editions between 1855 and 1900 (31) and provides the ur-text for most historically informed modern absinthe recipes.

For these reasons, a particular effort was made to secure as many different samples of preban Pernod Fils as possible; in total six are included in our analysis. It is worth noting that samples from the two largest Swiss based manufacturers, Edouard Pernod and Berger, were also included in the analysis.

Some General Notes on the Survival of Vintage Absinthe in the Modern Era. The majority of the unopened preban bottles of absinthe that have surfaced in recent times are representative of the most widely distributed and most respected marques. The bias of surviving examples toward the better margues can be explained not only by the fact that many of them were among the largest producers, but also by the circumstance that those who possessed the luxury of long-term suitable storage (e.g., wine caves) had the means to accumulate the better brands. It seems reasonable to assume that, upon the interdiction and disappearance of absinthe in Europe and the advent of World War I, those who were in possession of such bottles preserved them as keepsakes and mementos of a happier period. Many of these bottles were forgotten following the casualties of two wars and the passage of decades of time, which helps explain why some have remained preserved undisturbed in their cellars until the present day. In contrast, those dubious and short-lived marques, usually of Parisian origin, that represented the cheapest, most likely adulterated examples were purchased by those of lower socioeconomic status, in urban areas where storage space would be at a premium, and the prospects of long-term storage were thus far less likely. Cheaper brands were often sold only in cask (for the publican to dispense directly), precluding the possibility altogether of survival in bottle. The theoretical likelihood that some of these cheaper products contained significant concentrations of methanol, copper, antimony and/or other likely impurities and adulterants cannot be excluded, although it must be emphasized that no such samples have been discovered thus far.

Food Toxicological Evaluation Leading to Change of Food Policy. The relegalization of absinthe in Europe can be traced back to an evaluation of thujone by the Codex Alimentarius commission of the FAO/WHO in 1979 (51), followed by the Codex Alimentarius general requirements for natural flavorings published in 1985, which allow the use of thujone-containing flavorings up to certain maximum levels (i.e., 10 mg/kg thujone in alcoholic beverages above 25% vol and 35 mg/kg thujone in bitters) (20). The general requirements were introduced into the laws of many countries including the European Union (21) and Switzerland (52). The current proposal for amending the European law on flavorings and certain food ingredients with flavoring properties specifies a general maximum level of 35 mg/kg for all alcoholic beverages produced from Artemisia species, as there was an uncertainty in the past as to which of the Codex Alimentarius maximum levels should be applied to absinthe (53).

So far, the U.S. has not introduced the Codex Alimentarius recommendations about thujone into their law. However, a historical milestone was observed on March 5, 2007, when the U.S. government has approved the first genuine absinthe (Lucid, Viridian Spirits, Inc.) for U.S. distribution since 1912 (54). The seemingly insurmountable task of changing the views of the U.S. government with respect to the legality of absinthe was achieved largely on the strength of recently published peer reviewed studies on absinthe and thujone, e.g (4, 7, 14), as supporting evidence in the case for relegalizing absinthe. In consideration of these recent studies, the U.S. government has concluded that finished spirits that exhibit <10 mg/L thujone (i.e., the detection limit of the AOAC method (55)) effectively satisfy the "thujone-free" requirement in accordance with US 21 CFR 172.510 (56, 57). This stance was adopted in accordance with the Codex Alimentarius recommendation with respect to thujone content in finished spirits and represents not a change in the U.S. legal code but rather an acknowledgment that an indicated thujone content of <10 mg/L is far too minute to present any genuine public health concerns.

Given that five of the preban absinthes investigated in this study, would have passed the comparably strict U.S. standards, this seems to indicate that essential oil characteristics specific to the particular selection of herbs are at least as influential on the final content as are details of the distillation methods themselves. Logically this allows modern absinthes to be crafted and modeled after at least some original preban examples and recipes, but with the modern quality-controlled versions delivering more predictable thujone content, purely aimed at satisfying current regulatory requirements and having seemingly little significance otherwise. The previous view that special measures must be applied (e.g., extraction of the wormwood herb with supercritical carbon dioxide) as prerequisite to manufacture absinthe according to historical recipes (1) was clearly proven wrong.

The prevalence of the twin myths that preban absinthe generally contained thujone levels in the 260 mg/L range and that thujone in the quantities actually found in absinthe is either psychotropic or harmful in nature is hard to explain with reference to the available scientific research in the modern era. Besides the lack in chemical evidence about the toxicity of preban absinthe, the recent work of Luauté in studying the historical French sources (1860–1915) about absinthism proved that the epidemiological evidence is also completely missing to distinct this syndrome from general alcoholism (*58, 59*).

Today it seems a substantial minority of consumers *want* these myths to be true, even if there is no empirical evidence that they *are*. These consumers seem to feel that absinthe, in view of its fabled and exotic reputation, *ought* to be dangerous, even in the absence of evidence that it *is*. It is to be hoped that this paper will go some way to refuting at least the first of these myths, conclusively demonstrating that the thujone content of a representative selection of preban absinthe, including the largest and most popular brands, fell within the modern EU limit of 35 mg/L.

Supporting Information Available: Photographic documentation of the sampling process. This material is available free of charge via the Internet at http://pubs.acs.org.

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