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Gas Chromatography–Mass Spectrometry Analysis of Alaska Yellow-Cedar Extractive Components

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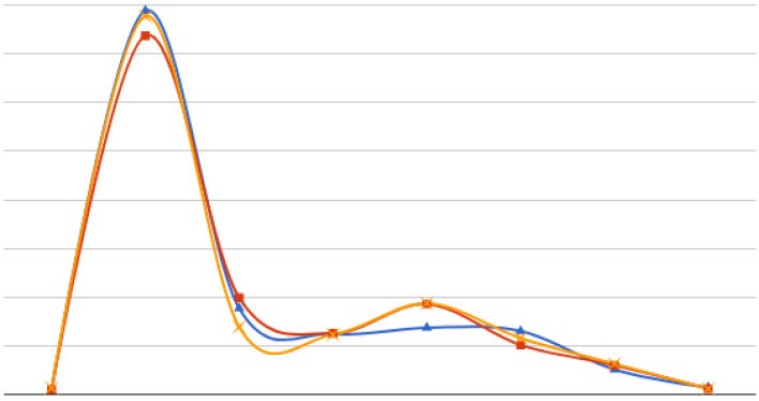

Abstract

Alaska yellow-cedar (*Cupressus nootkatensis*) is a softwood tree species native to the Pacific coast of Canada and is known to contain various biologically active extractive components. Extractives are nonstructural chemical compounds found within wood that are primarily responsible for the durability of the tree itself and can, in some species, fend off attacks from microbes, insects, and other agents. Durability in wood often translates to biological activity in the human body, and some extractive compounds have already been identified as being biologically active. Extractives of Alaska yellow-cedar were separated using liquid–liquid fractionation and were identified using gas chromatography–mass spectroscopy (GC–MS). Vial B solvent mixture polarity separated 50% of the sample and had more compounds identified than the other fractions. The separated samples in vials F and G each had only one compound identified, and vial H had no compounds identified. Multiple compounds were identified in the other fractions that exhibit biological activity, such as carvacrol, nootkatone, α-cadinol, androsta-1,4,6-trien-3-one,17-hydroxy-(17β), and falcarinol. Further investigation on the vials with one or less compounds will be analyzed using instrumentation with more sensitivity and resolving power to identified trace compounds.

Keywords: extractives, liquid-liquid fractionation (LLF), gas chromatography–mass spectrometry (GC–MS), polar–nonpolar compounds, analyte, supernatant, solvents, percentages

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Gas Chromatography–Mass Spectrometry Analysis of Alaska Yellow-Cedar Extractive Components

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Introduction

The extractives of wood are like antibodies found in the human body in that their concentrations increase when the tree is under attack from threats such as fungi and termites (Taylor et al. 2002). Although they do not provide structural support to the tree, extractives are the main factor determining the degree of durability of different types of wood (Kirker et al. 2013). Alaska yellow-cedar (*Cupressus nootkatensis*), a softwood found primarily on the western coast of Canada and the Pacific Northwest coast of the United States, is commercially popular because of its durability, strength, and straight grain (Khasawneh 2003). Carvacrol and nootkatone were shown to be major components of Alaska yellow-cedar extractives (Khasawneh 2003). Carvacrol is a phenolic compound that has been shown to improve durability (Moore et al. 2013) and exhibit different types of biological activity (Wang et al. 2020).

Solid phase extraction (SPE) was previously a popular method for fractionating wood extractives, but the use of SPE cartridges can lead to significant loss of sample and skewed results. To address this issue, one goal of this research project was to develop an efficient method for liquid–liquid extraction (LLE) that would successfully separate extractives into smaller fractions while also reducing sample loss. If sample loss were reduced or eliminated altogether, new compounds could potentially be identified that were previously being lost in the stationary phase. In addition, gas chromatography–mass spectroscopy (GC–MS) is a useful method of identifying unknown compounds in solution. For GC/MS to give cleaner results with no broadening of peaks, the number of compounds in each sample had to be reduced, hence the need for fractionation. The main aim of this study was to identify all compounds found in the extractives of Alaska yellow-cedar using LLE and GC–MS analysis.

Experimental Methods

Sample Preparation

A sample of Alaska yellow-cedar was ground and Wiley milled to 40 mesh. Soxhlet extraction was performed using a 9:1 acetone:water mixture for 10 h. Excess solvent was then evaporated using a rotary evaporator, and the remaining extractives were stored in a vial in the freezer.

Liquid–Liquid Extraction

Using the methods of Willfor et al. (2015), acetone and hexane were proposed as the polar and nonpolar solvents, respectively, used for this experiment. Mixtures were prepared in the following ratios (acetone:hexane): 10:90, 20:80, 30:70, 45:55, 60:40, 80:20, 100:0. Approximately 100 mg of Alaska yellow-cedar extractives was added to a sample vial; 3 mL of the first solvent mixture (10:90) was added to the extractives, and the vial was sonicated, vortexed, and placed on the bench to allow the sample to settle. After the sample settled, the supernatant was decanted into a 7-mL vial. The sample was washed with 9 mL of each ratio of solvent mixture, and each wash was placed in a separate vial. Three replicates were performed on consecutive days.

Gas Chromatography–Mass Spectrometry

Analysis of the extractives was performed using an HP 5890 Series II Plus Gas Chromatograph (HP Inc., Palo Alto, California) equipped with a HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness). The column temperature was initially 60 °C for 1 min, then gradually increased to 300 °C at 10 °C/min and held there for 3 min. Extractives were dissolved in 500–1,000 µL of 1:1 (v/v) acetone:methanol, depending on weights, then pipetted into 1,000-µL GC vials. Vials of dissolved sample were then injected automatically into the instrument.

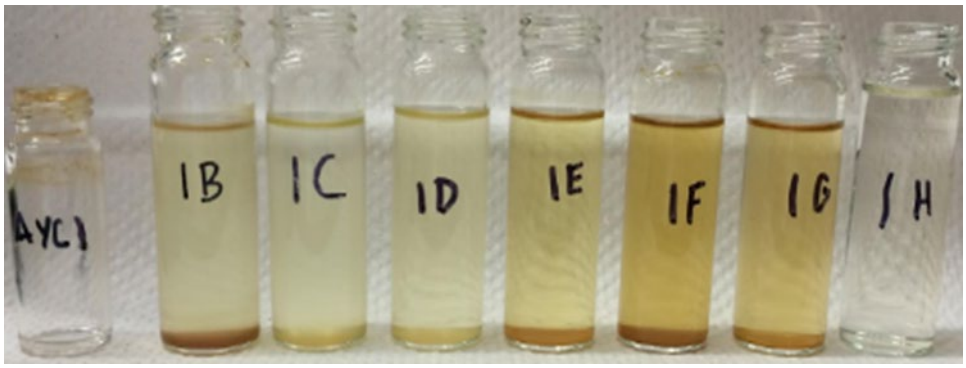

Figure 1—Supernatant vials after the first replicate was completed. Solvents become more polar from left to right. Residual sample is at the far left in the vial labeled AYC1.

Results and Discussion

Visual Observations

The solid sample had a dark yellow color and all the washes, except from the pure acetone, exhibited yellow tints of various intensities. The samples were washed with 9 mL of solvent mixture for each vial. Results from previous trials suggested that 9 mL was sufficient to dissolve the analyte. Figure 1 shows the solutions after the first completed trial. The color intensity faded with each 3-mL increment decanted in each vial. The first three vials, B, C and D, were pale yellow. The color intensity indicated that these samples were not concentrated. Vials E, F, and G were a dark shade of yellowish brown, which should indicate that these samples were highly concentrated. Vial 1F had the most intense shade of yellow, but it only weighed 9.10 mg of sample. In theory, the solution in vial 1B should have been the darkest as it contained approximately five times the amount of sample than vial 1F. This was not the case. The polar fractions (vial 1F) were darker and had more intense color but were not concentrated. Nonpolar fractions (vial 1B) were not intense in color but were more concentrated. Vial H was pure acetone, and the solvent solution was clear. The sample was completely distributed and fractionated into each vial until the sample vial was empty.

Polarity Fractionation

Compounds identified from the fractionations are described in the Appendix. Table 1 separates compounds according to polarity from the LLE. The GC–MS identified the most compounds from vial B, in which they were primarily dissolved in hexane. Each fractionation had similar compounds in vials B, C, and D, which is expected because the polarity of the solution slightly changed for each vial.

Most of the sample was dissolved in nonpolar solution, which is expected in softwoods. Figure 2 shows the average weight percentage dissolved in each solution along with the 95% confidence interval, accounting for any variability. The slight increase in dissolved analyte in the 45:55 solution shows that some compounds near the middle of the polarity spectrum, possibly phenols, are found in the extractive sample along with nonpolar compounds. Because hardly any sample was left by the pure acetone wash; this could suggest that there are no extractive components with polarities similar to pure acetone.

Vials B, C, and D contain mostly nonpolar solvents, whereas vials F, G, and H contain mostly polar. Vial E contains solvent close to 50% polar and nonpolar, so it was excluded when considering polar vs nonpolar fractions. Nonpolar extractive content accounts for 74.4% of the total sample, whereas polar content accounts for only 13.0%. This is consistent with previous findings (though using a different method) that softwood tree species have less polar extractives than do hardwoods (Moore et al. 2015).

Gas Chromatography–Mass Spectrometry

GC–MS data in Table 1 showed a wide range of compounds identified in the supernatants. There appeared to be a high concentration of one specific phenol, carvacrol, found in vial B, which is consistent with the findings of Khasawneh et al. (2011). It is a major compound found in aromatic plants such as origanum, thymus, and coridothymus species. This compound is used to treat human skin diseases. Carvacrol has been tested and shown to exhibit antiviral activity, inhibiting the HS virus proliferation process (Wang et al. 2020). Nootkatone, an abridged name for the compound titled 2(3H)-Naphthalenone, 4,4a,5,6,7,8 hexahydro-4,4a-dimethyl-6-(1-methylethenyl), was found in high concentrations in vials B and C and has been proven to be a successful insecticide, possibly contributing to Alaska yellow-cedar's durability (Schrader and Bohlmann 2015). GC–MS spectra and chromatograms of both carvacrol and nootkatone are shown in Figure 3. There has also been evidence that nootkatone could potentially act as an acetylcholinesterase inhibitor (Curini et al. 2006), which have been used in multiple pharmaceuticals to treat conditions such as Alzheimer's disease, Parkinson's disease dementia, and other neurological disorders (Colovic et al. 2013). The compound α-cadinol, found in vial B, has shown promise as an antifungal agent and also helps to prevent damage to the liver (Ho et al. 2011, Tung et al. 2011), which makes it a good candidate for pharmaceuticals related to

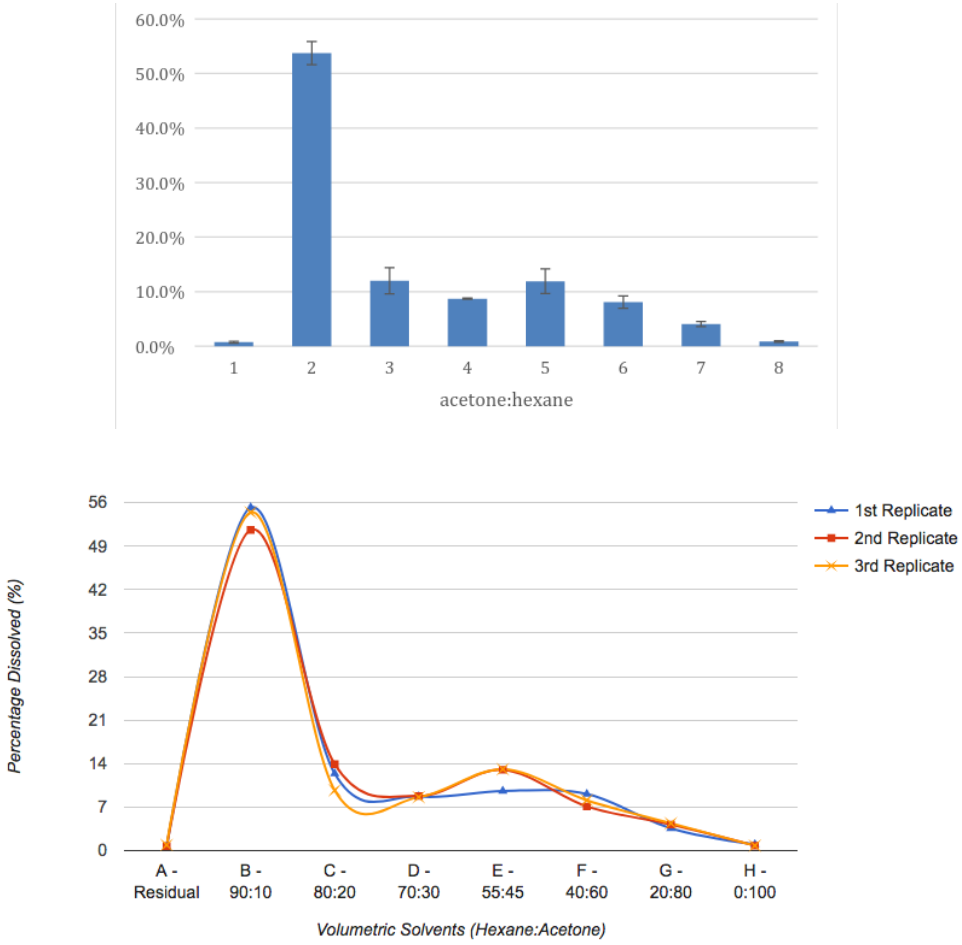

Figure 2—Average percentages of Alaska yellow-cedar sample left in each vial. Included in the bar graph are 95% confidence intervals.

liver protection. The compound 5,6-azulenedicarboxaldehyde, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-, (3aα,8α,8aα)-(-), better known as velleral, was found in vial B and is used by mushrooms as a defense system (Sterner et al. 1985). Androsta-1,4,6-triene-3,17-dione (ATD), found in vial E, is classified as an aromatase inhibitor that reduces estrogen production and in turn increases the concentrations of steroids, such as testosterone, in the body (Kwok et al. 2015). Because of this, ATD has been used to treat breast cancer and is also a popular supplement for bodybuilding (Parr et al. 2009). Falcarinol, found in vial G, has been shown to inhibit cancer tumor growth and is currently being used as a natural anticarcinogenic agent (Zaini et al. 2015). Chemical structures of some of the compounds listed in Table 1 are provided in the Appendix (Table 2).

Conclusion

Alaska yellow-cedar is a highly durable and very popular wood for outdoor and indoor projects. It is known for its long-lasting resistance to environmental elements, which results in part from the large proportion of nonpolar compounds in its extractives. Based on this study, LLE using acetone and hexane is a good way to separate various compounds of Alaska yellow-cedar. Limited compounds containing biological activity were identified, including carvacrol, nootkatone, α-cadinol, androsta-1,4,6-trien-3-one, 17-hydroxy-(17β), and falcarinol. Further investigation will be done on vials F, G, and H samples utilizing GC×GC TOFMS (time-of-flight mass spectrometry).

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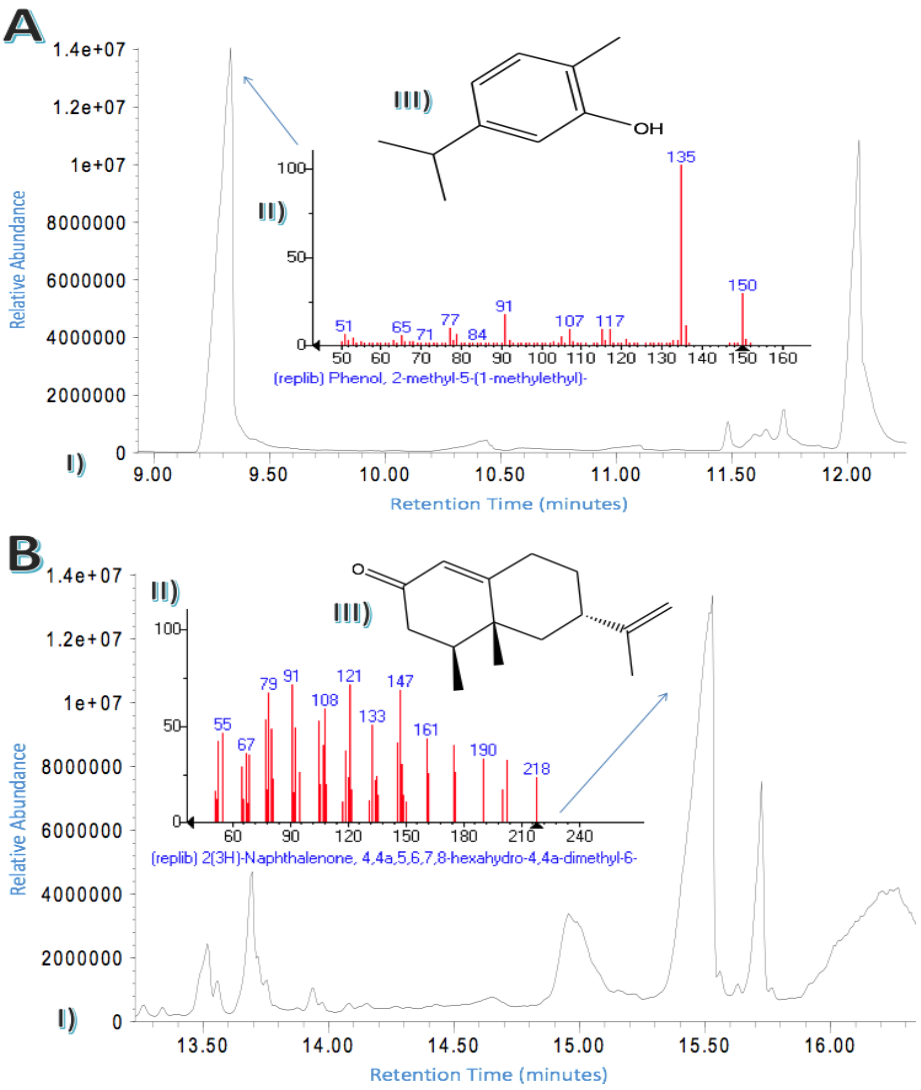

Figure 3—GC–MS data: (I) chromatograms, (II) spectra, and (III) chemical structures similar to (A) carvacrol and (B) nootkatone. These compounds were identified in vial B of Alaska yellow-cedar extractions. (Chemical structures were generated using ChemDraw Ultra 7.0.)

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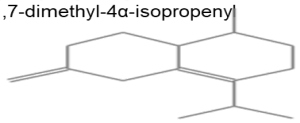
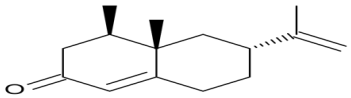
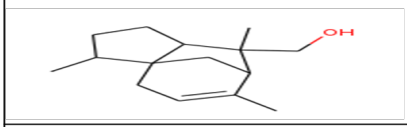
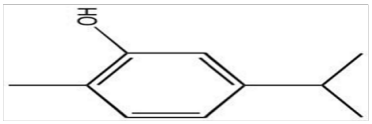
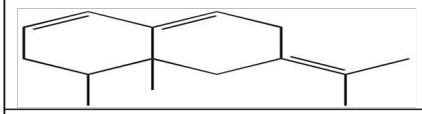
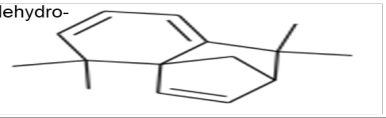
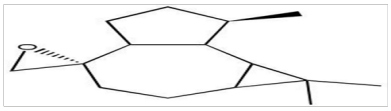
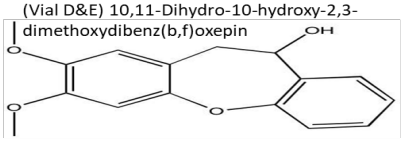
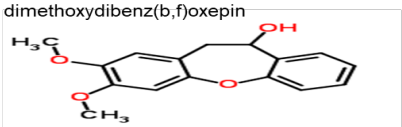
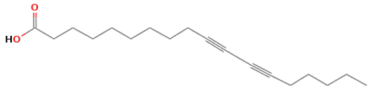
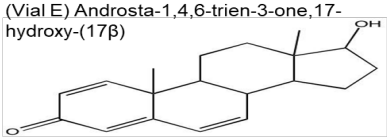
Appendix—Compounds Identified from Fractionations

Table 1—List of identified compounds of Alaska yellow-cedar found in each vial by GC–MS

Vial	Compound	Retention time (min)	Formula
B	Carvacrol	9.325	$C_{10}H_{14}O$
	β-Vatirenene	12.050	$C_{15}H_{22}$
	Tau-muurolol	13.524	$C_{15}H_{26}O$
	α-Cadinol	13.689	$C_{15}H_{26}O$
	Cedren-13-ol,8	14.984	$C_{15}H_{24}O$
	2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-Methylethenyl) (nootkatone)	15.516	$C_{15}H_{22}O$
	γ-Gurjunenepoxide	15.724	$C_{15}H_{24}O$
	Bicyclo[4.4.0]dec-6-en-9β-ol,1,7-dimethyl-4α-isopropenyl	16.271	$C_{17}H_{26}O_2$
	5,6-Azulenedicarboxaldehyde,1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-,(3aα,8α,8aα)-(-)	17.055	$C_{15}H_{20}O_2$
C	Isolongifolene,4,5,9,10-dehydro-	11.710	$C_{15}H_{12}O$
	γ-Gurjunenepoxide-(2)	14.104	$C_{15}H_{24}O$
	2(3H)-Naphthalenene,4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-Methylethenyl)	15.391	$C_{15}H_{22}O$
	Aramodendrenoxide-[2]	16.671	$C_{15}H_{24}O$
	Dihydrofuran-2-one,4-(3.4-dimethoxybenzyl)-3-(4-hydroxy,3-methoxybenzyl)	26.047	$C_{21}H_{24}O_6$
D	γ-Gurjunenepoxide-(2)	14.180	$C_{15}H_{24}O$
	10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f)oxepin	22.823	$C_{16}H_{16}O_4$
	Dihydrofuran-2-one,4-(3,4-dimethoxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)	26.015	$C_{21}H_{24}O_6$
E	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	14.797	$C_{15}H_{22}O$
	Methyl 4,6-tetradecadiynoate	15.934	$C_{15}H_{22}O_2$
	10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f)oxepin	22.793	$C_{16}H_{16}O_4$
	Androsta-1,4,6-trien-3-one,17-hydroxy-(17β)	24.210	$C_{19}H_{24}O_2$
	Dihydrofuran-2-one,4-(3,4-dimethoxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)	26.614	$C_{21}H_{24}O_6$
F	10,12-Octadecadiynoic acid	14.897	$C_{18}H_{28}O_2$
G	Falcarinol	12.751	$C_{17}H_{24}O$
H	(No detectable peaks)		

Table 2. Chemical structures for some of the compounds listed in Table 1

(Vial B) Bicyclo[4.4.0]dec-6-en-9β-ol,1,7-dimethyl-4α-isopropenyl 	(Vial B) nootkatone 
(Vial-B) Cedren-13-ol,8 	(Vial B) Carvacrol 
(Vial B) β-vatirenene 	(Vial C) Isolongifolene,4,5,9,10-dehydro- 
(Vial C) Aramodendrenoxide-[2] 	(Vial D&E) 10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f)oxepin 
(Vial E)10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f)oxepin 	(Vial F) 10,12-Octadecadiynoic acid 
(Vial E) Androsta-1,4,6-trien-3-one,17-hydroxy-(17β) 	(Vial G) Falcarinol