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Contaminants Present in Materials Commonly Used to Purify Plant Extracts for Hormone Analysis[1]

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Abstract. **Sensitive analytical techniques such as gas chromatography or combined gas chromatography-mass spectrometry, detect not only plant hormones, but chemical contaminants as well. Thorough cleaning of solvents and chromatographic materials used for extracting plants as well as identification of the contaminants present will assist in resolving the contaminants from hormones present in the same extract.**

Bioassay of extracts permits the detection of submicrogram quantities of plant hormones. Contaminants present in solvents or chromatographic supports are seldom a problem as they are usually biologically inactive at the concn encountered, although exceptions have been reported (2). When gas chromatography (GC) is used for analysis, however, contaminants can be readily detected and thus can present problems. For example, the plasticizer acetyl tri-n-butyl citrate is often detected in plant extracts (1). Further, n-alkanes can be introduced into extracts when Parafilm is used to cover glassware containing plant samples (3). In this report we indicate the wide-spread occurrence of other artifacts, and identify several of the most abundant.

Standard commercially available solvents were redistilled on a fractionating column prior to use and stored in glass bottles with Teflon-lined lids. The following chromatographic materials were used: "Silicar" TLC 7G (Mallinckrodt Chemical Co.); 0.25 mm precoated, aluminum-backed TLC plates of silica gel 60 without fluorescent indicator, (Merck and Co.); polyvinylpyrrolidone (PVP) polyclar AT (GAF Corporation); and instant thin-layer chromatography (ITLC) (Gelman Products). The solvents and chromatographic materials were analytical grade and were used in the same manner as when purifying plant extracts.

On evaporating the solvent, the residues were dissolved in methanol and methylated with ethereal diazomethane. The methylated (Me) samples were either dissolved in ethyl acetate and analyzed by GC directly, or dissolved in dry pryridine and silylated by adding hexamethyldisilazane:trimethylchlorosilane: pyridine (2:1:2, v/v/v), resulting in methyl ester trimethylsilyl ether (MeTMS) derivatives for GC.
derivatives for GC.

The Me and MeTMS derivatives were chromatographed on a Pye 104 gas chromatograph using 2% SE 33 on Gas Chrom Q (80-100 mesh) packed in 153.4 cm x 3.5 mm i.d. glass columns. Column temp was programmed from 180° to 225°C at 3°/min, with a nitrogen flow rate of 60 ml per min. Gas chromatography-mass spectrometry (GC-MS) of MeTMS derivatives was done on an A.E.I. MS30 coupled to a Pye 104 GC through a silicone membrane which prevents helium from entering the MS. The 213.4 cm x 2 mm i.d. glass column for GC-MS was packed with 2% SE33 on Gas Chrom Q 980-100 mesh), and the helium flow rate was 25 ml per min. Mass spectra were obtained at 24 eV with a source temp of 210° and a separator temp of 190°. The MS were recorded at 6.5 sec per

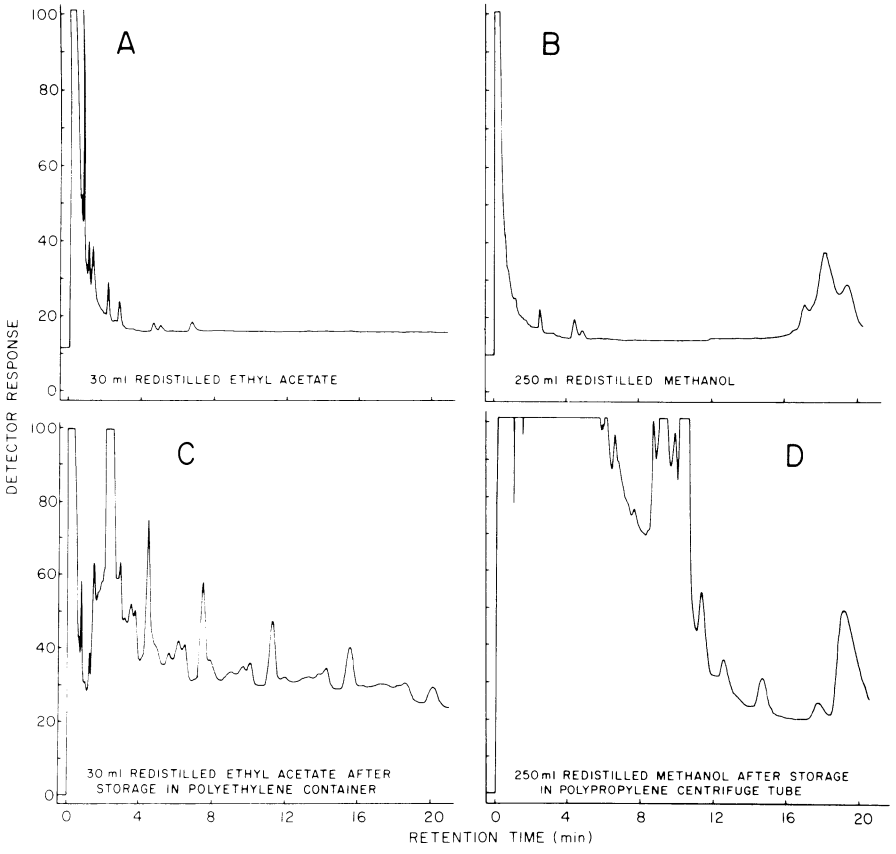

Fig. 1 GC traces of residues of solvents before (A and B) and after (C and D) 4 days of storage in synthetic containers.

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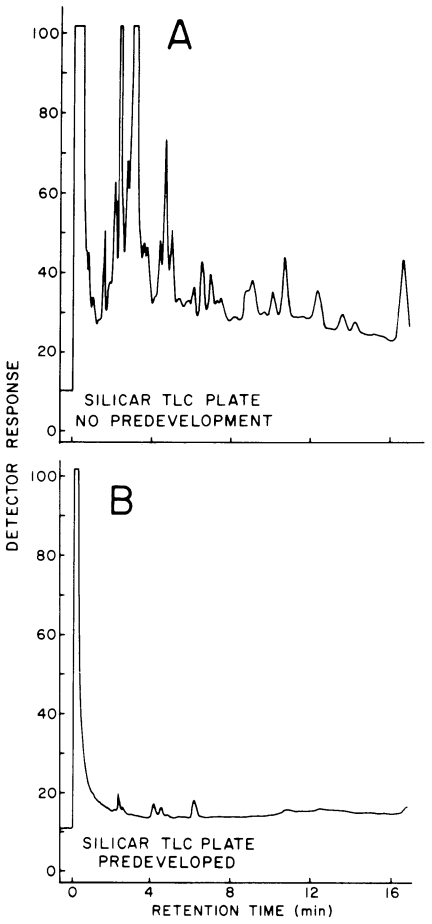

Fig. 2. GC traces of residues of wet ethyl acetate eluates of 8 x 20 cm sections of silicar TLC plates A) before and B) after pre-development with wet ehyl acetate.

decade and processed online by a Line 8 computer. All compounds listed were identified by comparison with authentic standards.

When small volumes of redistilled ethyl acetate or methanol are concn and the residues methylated and GC'd, contaminants are evident (Fig. 1A and B). Storage of these solvents in polyethylene or polypropylene containers for 4 days results in high levels of additional contaminants (Fig. 1C and D).

Commonly TLC is used to purify extracts containing plant hormones. We were interested to determine the presence of possible contaminants in readily available TLC materials. Soluble materials from 'Silicar' TLC plates 8 x 20 cm were eluted with water-saturated ('wet') ethyl acetate either before or after pre-development in the same solvent. The filtered eluates were concd methylated, and GC'd. Many contaminants were eluted from the untreated plate (Fig. 2A), but pre-development in wet ethyl acetate markedly reduced their levels (Fig. 2B). As expected, results differed with different solvents. Pre-development in wet ethyl acetate *reduces* contaminants in aluminum-backed TLC, while *increasing* those in ITLC (data not shown). In the latter case, pre-development may desorb chemicals which are subsequently solubilized by the eluting solvent.

The examples which follow illustrate the importance of checking for possible contaminants present in solvents and chromatography materials. An aluminum-backed TLC plate was pre-developed in wet ethyl acetate, developed with 15 ethyl acetate:5 chloroform:1 acetic acid (v/v), and and 8 x 20 cm zone was eluted with wet ethyl acetate. The eluate was concd and the residue was methylated and silylated. GC-MS (Fig. 3A) revealed the presence of 5 fatty acids, 5 alkanes, 3 phthalate derivatives, dehydroabietic acid, and a polyisoprenoid, in addition to several unidentified compounds.

PVP, which can be used to partially purify plant extracts (4), also contains contaminants. We mixed 2 g of PVP with 75 ml phosphate buffer, pH 8.0 and the suspension then filtered. The filtrate was partitioned 3 times against 25 ml ethyl acetate. The ethyl acetate layer was discarded, and the remaining phosphate buffer phase was adjusted to pH 3.0 and partitioned 3 times against 25 ml ethyl acetate. The acidic ethyl acetate phase was evaporated to dryness, and the residue methylated, and silylated. Peaks corresponding to 5 fatty acids, 2 phthlalate derivatives, acetyl-tri-n-butyl citrate, a polyisoprenoid, and several unidentified compounds were observed (Fig. 3B).

Complete removal of extraneous artifacts was found to be impractical. However, their levels should be minimized by rigorous purification of solvents and materials used at each step in the fractionation of extracts. Otherwise, these introduced impurities will both hamper identification of endogenous compounds and lead to incorrect deductions as to plant composition. On the latter point, a more serious difficulty may be the accidental contamination of plant extracts by standard compounds, e.g., GA_3, in use in the laboratory (5). With the increasing sensitivity of GC-MS, negligence may lead to spurious identification of such contaminants as naturally occurring components of the extract.

An additional technique useful for resolution of mixtures is called selective ion current monitoring (SICM). In this MS procedure, one focuses the instrument on major characteristic ions of the

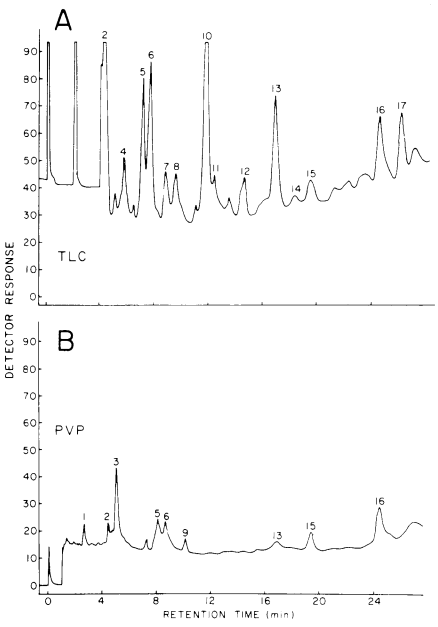

Fig. 3. Total ion current trace of residues of A) an aluminum-backed TLC plate pre-developed in wet ethyl acetate before development in ethyl acetate:chloroform: acetic acid (15:5:1, v/v/v), and B) the acid ethyl acetate fraction of a phosphate buffer eluate of PVP. Numbers on the total ion current trace indicate the following compounds, identified by GC-MS: 1-myristate; 2-palmitate; 3-fatty acid; 4-alkane; 5-oleate; 6-stearate; 7-fatty acid; 8-alkane; 9-acetyl-tri-n-butyl-citrate; 10-dehydroabietic acid; 11-alkane; 12-alkane plus phthalate; 13-di-octyl-phthalate; 14-alkane; 15-di-nonyl-phthalate; 16-polyisoprenoid; and 17-ω-hydroxydocosanoic acid.

compound of interest (6). The resultant combination of GC retention times and SICM helps factor out extraneous artifacts.

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