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Suicidal yew leave ingestion – Phloroglucindimethylether (3,5-dimethoxyphenol) as a marker for poisoning from Taxus baccata

Frank Mußhoff, Bernhard Jacob, Cornelia Fowinkel, and Thomas Daldrup

Institute of Legal Medicine, Heinrich Heine University Düsseldorf, Moorenstrasse 5, D-40225 Düsseldorf, Germany

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Summary. In a case of suicide in a depressive 19-year-old man with considerable ingestion of new leaves, resorption of yew ingredients could be demonstrated. The main substance could be identified as 3,5-dimethoxyphenol, the aglycone of taxicatine, which is a typical ingredient of yew leaves. 3,5-dimethoxyphenol was demonstrated in harvested yew leaves, stomach content and cardiac blood of the victim. Structure confirmation was achieved by means of HPLC, UV, GC-MS, IR and ^{1}H-NMR spectroscopy. None of the Taxus alkoids could be identified. The components detected by TLC have not yet been identified. The results demonstrate that 3,5-dimethoxyphenol can be used as a marker in cases of intoxication by yew ingredients.

Key words: Fatal intoxication – Suicide – Taxus/yew ingredients – 3,5-dimethoxyphenol

Zusammenfassung. Im Rahmen der Untersuchung eines Suizides eines depressiv verstimmten 19jährigen Mannes mit einer großen Menge von Eibennadeln konnte die Resorption von Eibeninhaltsstoffen nachgewiesen werden. Als Hauptsubstanz fand sich 3,5-Dimethoxyphenol, das Aglykon des Taxicatins, ein eibentypischer Inhaltsstoff. Es wurde in geernteten Eibennadeln, im Magen und im Herzblut des Opfers nachgewiesen. Die definitive Strukturbestätigung des aufgefundenen 3,5-Dimethoxyphenols erfolgte durch HPLC, GC/MS, UV-, IR- and ^{1}H-NMR-Spektroskopie. Es konnten keine vom Taxin ableitbaren Alkaloide identifiziert werden. Dünnschichtchromatographisch nachgewiesene Inhaltsstoffe konnten nicht zu Alkaloidbruchstücken zugeordnet werden. Das nachgewiesene 3,5-Dimethoxyphenol kann als Leitsubstanz für den Nachweis von Intoxikationen mit Eibenbestandteilen herangezogen werden.

Correspondence to: B. Jacob

Schlüsselwörter: Tödliche Intoxikation – Suizid – Taxus/Eiben Inhaltsstoffe – 3,5-Dimethoxyphenol

Introduction

The toxic effects of the ubiquitious yews and their leaves have been known since ancient times. Accidental ingestion, especially of berries, in smaller amounts occur quite often in children mostly with mild signs of intoxication [31, 32]. The ingestion of leaves in larger amounts may cause serious intoxication [2, 4, 6, 9, 18, 29, 37] or death in man and especially in live stock [1, 3, 23, 28]. However deliberate intoxication for suicidal purposes have been reported in only a limited number of cases [5, 6, 9, 11, 21, 34, 35, 37].

Yews contain a variety of taxine-derived alkaloids, taxane-derived compounds and several glycosides [13, 16, 17, 26, 36]. The cases of intoxication reported so far lack a clear toxicological proof of the resorption of yew ingredients. Only Frohne and Pribilla [11] could demonstrate an extractable substance in the stomach contents and in the liver of a suicide victim that was also present in harvested yew leaves, however a definite determination of the structure of the substance was not carried out.

Recently we had the opportunity to examine a suicide victim after yew leaf ingestion.

Case report

A depressive 19-year-old man (3/91) was found by his friends in a remote cellar after having disappeared 2 days earlier.

The dead man was lying on a couch partly undressed. An empty teapot with fragments of brown-greenish leaves on the sides was found on the floor. Near the teapot brewed and pressed leaves

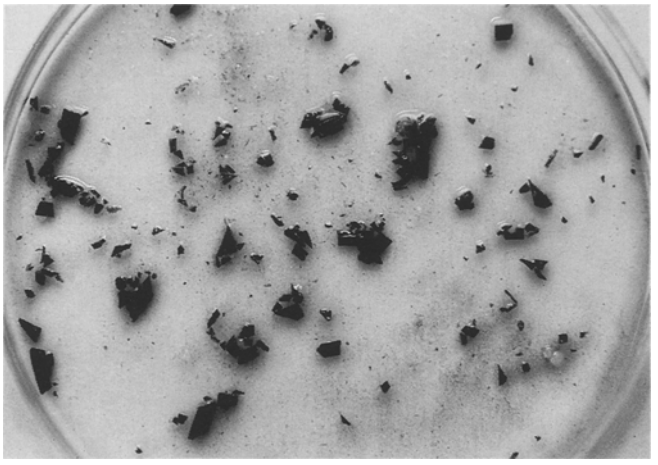


Fig. 1. 2 ml of stomach contents from the victim in a petri dish

were piled up with a teaspoon on the carpet. The cellar was free of signs of vomitting or diarrhoea, however a toilet was nearby.

The forensic autopsy, carried out one day after discovery, revealed fragments of greenish needle-like leaves in the mouth, esophagus and stomach as well as in the intestines but not in the rectum (see Fig. 1). A separation of leaves and gastric fluid revealed that 30% of the 500 g stomach contents were needles (wet mass). All organs were markedly congested. The bronchial epithelium was markedly inflammed without signs of aspiration. Significant diseases or inflammation of the gastrointestinal tract were not observed.

Materials and methods

Histological examination

Specimens of all organs were taken at autopsy, fixed in 5% formalin, embedded in paraplast and stained with H & E, Elastica van Gieson, Berlin blue and alzian blue according to standard laboratory procedure.

Analytical methods

For further examination 1 g of cardiac blood, stomach contents and yew leaves were extracted 3 times with 3 ml of ethyl acetate. The combined organic phases were evaporated under nitrogen at 50°C. The evaporates were dissolved in methanol: Stomach contents in 500 µl, cardiac blood in 50 µl and yew leaves in 100 µl.

The following chromatographic procedure for the analysis of unknown toxicological substances [7] was carried out on the stomach contents, liver, cardiac and femoral blood:

Thin layer chromatography (TLC) was performed with an ethyl acetate/methanol/ammonia (85:10:5) solvent on 10 cm silicagel glass plates F254 (Merck). Detected substances were characterized by their corrected retention (RF_c) values in relation to the retention of 2 internal standards [8, 12].

High performance liquid chromatography (HPLC) screening was performed with either an acetonitrile/water solvent (LC I) or with an acetonitrile/phosphate buffer (LC II). LC I was an aqueous solution of 31.2% acetonitrile in water (w/w). LC II was a solution of 31.2% acetonitrile in aqueous buffer (w/w). The buffer contained 4.8 g of 85% orthophosphoric acid and 6.66 g of KH_2PO_4/l water adjusted to pH 2.3. For both solvents Kontrosorb 10 RP18 columns (250 × 4.6 mm i.d.) were used. For LC I a Perkin Elmer LC 75 UV spectrophotometric detector working at 220 nm with a Sigma 10 integrator was applied. With the LC II solvent a Perkin Elmer LC-480 auto scan diode array detector with a 16 mm cell was used as detecting system.

Mass spectrometry (MS) following gas chromatography (GC) was carried out on a Hewlett Packard HP 5970 mass selective detector and a Hewlett Packard HP 5890 A gas chromatograph. Injection (split/splitless, split open after 2 min) was executed at an injection temperature of 280°C. The carrier gas was helium at a pressure of 40 hPa. The column was a fused silica capillary Ultra 1 column (12 m × 0.2 mm i.d.). Chromatography was achieved with a temperature gradient program: 40°C for 2 mins, linear ramp to 100°C (40°C/min) followed by a linear ramp to 300°C (10°C/min) and a final plateau held for 5 mins.

IR spectrometry of the KBr pelletted substance was performed on a Perkin Elmer 1420 ratio recording spectrometer.

^{1}H-NMR spectrometry was executed on a Varion VXR 300 at 300 MHz. The fractionated substance was diluted in deuterochloroform with tetramethylsilane as internal standard.

The blood alcohol concentration was determined by GC and by the alcohol dehydrogenase method (ADH). Measurements were carried out in duplicate and in accordance with the guidelines of the German Federal Office of Health (Bundesgesundheitsamt) for the forensic measurements of blood alcohol concentrations.

For GC measurement a Multifract F 40 (Perkin-Elmer) gas chromatograph was used equipped with a 1.8 m steel column of 0.3175 mm internal diameter. The column was packed with 15% carbowax 1500 on chromosorb W NAW, 80–100 mesh. Detection system was a flame ionization detector (FID). The carrier gas was N_2 with a column head pressure of 150 kPa. The injection time was 3 s, the temperature of the oven was 80°C. FID, Injector and transfer line temperature was 160°C, water bath temperature was 63°C. For GC measurement 50 µl of serum was added to an internal standard solution of t-butanol and measured automatically after temperature equilibration.

For ADH measurements 50 µl of serum were deproteinized with 1000 µl of 3.3% perchloric acid. The liquid phase was then measured on an Epos 5060 (Eppendorf) analyzer calibrated with external standards.

Results

Histological findings

Histological examination revealed a marked and sometimes severe congestion of the organs as observed macroscopically.

Cerebellar hemispheres showed little loss of purkinje cells. All areas of the central nervous system were characterized by signs of moderate neuronal damage, especially in the hippocampal region.

The lungs demonstrated massive desquamation of alveolar epithelium. There were no iron deposits but large amounts of brownish pigment were found in the alveolar macrophages. There were moderate peribronchiolar lymphocytic infiltrations as well as a moderate increase of bronchiolar muscle layers and epithelial thickening. Several of the bronchioli were occluded by mucus and desquamated ciliated columnar epithelial cells.

Cardiac muscle demonstrated little to moderate vacuolar degeneration, which was marked in the atrioventricular node.

Toxicological findings

TLC of aliquots of the stomach content revealed 2 Dragendorff positive spots with corrected retention val-

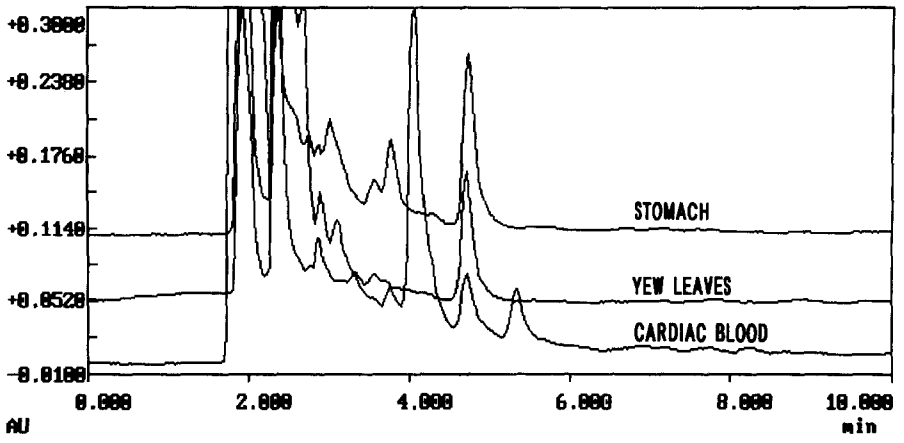


Fig. 2. Superimposed HPLC chromatograms of yew leaves, stomach content and cardiac blood extracts of the victim in the LC II solvent system at 220 nm, time versus absorption units

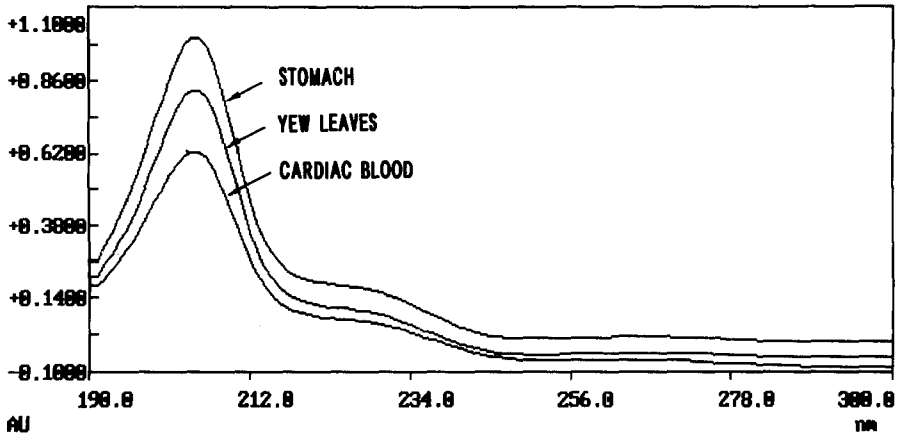


Fig. 3. Superimposed UV spectra (at 4.710 min) of yew leaves, stomach content and cardiac blood extracts of the victim in the LC II solvent system, wave length versus absorption units

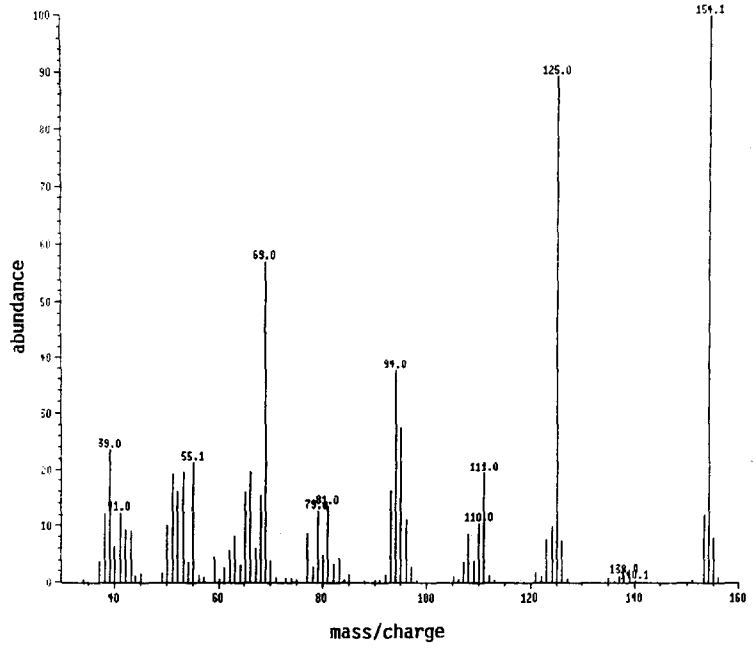


Fig. 4. Mass scan of the main peak of the GC chromatogram at 9.3 minutes of the yew leaves extract, mass/charge units versus abundance

ues (RF_c) of 66 and 76. The spots were removed and examined by MS but could not be matched to taxine derived molecules [11, 13–17, 22, 25, 26, 36].

HPLC screening of the stomach contents resulted in a chromatogram with different unknown substance peaks. Comparison of repeated extractions of yew leaves (triple extraction of dried leaves with ethyl acetate) with the stomach contents demonstrated a very high chromatographic correlation. There was a good correlation in HPLC as well as in the UV spectrum of the main (4.7

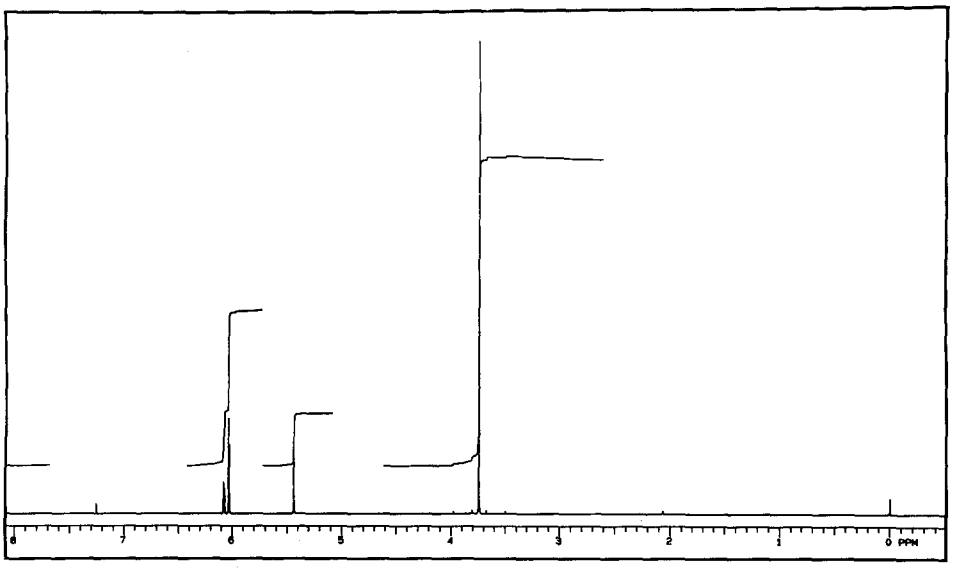


Fig. 5. ^{1}H-NMR spectrum at 300 MHz of the fractionated HPLC main peak diluted in deutero-chloroform with tetramethylsilane (TMS) as internal standard. The following chemical shifts (δ) were recorded in ppm in relation to TSM: δ: 3.75, s, 6H, OCH_3; δ: 5.44, s, 1H, OH; δ: 6.03, d, 2H; δ: 6.08, t, 1H. This confirms the supposed IR and GC-MS derived structure of the unknown substance as 3,5-dimethoxyphenol

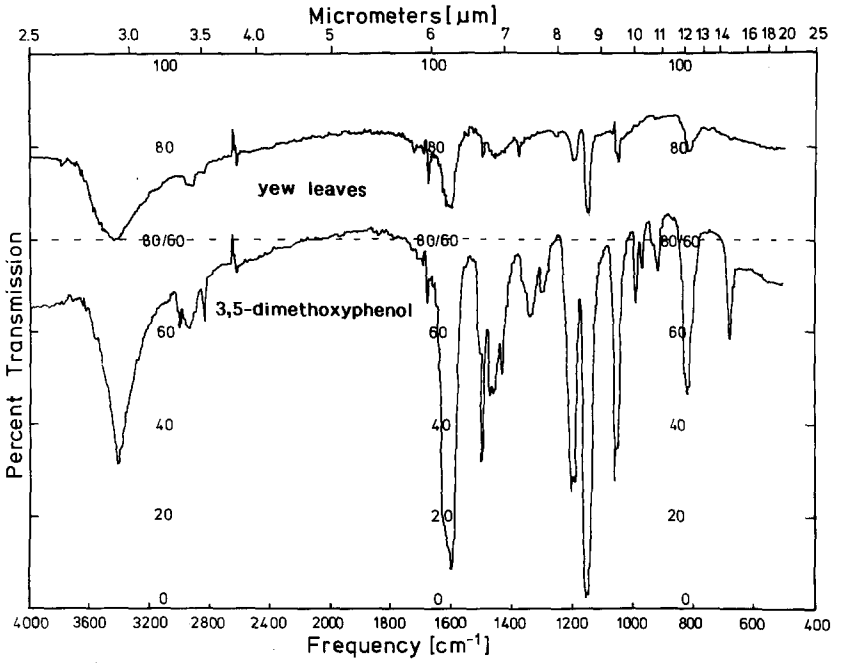
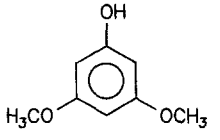


Fig. 6. Superimposed IR spectrum of 3,5-dimoxyphenol and yew leaves extract gained by HPLC fractionation, wave length and frequency versus transmission

min) peak. The ingredient could also be demonstrated in cardiac blood, however, the correlation was weaker (Figs. 2 and 3).

GC/MS examination of yew extract revealed a main peak at 9.3 mins with a major mass peak of 154 mass units (Fig. 4). To gain enough material of the main substance peak for identification 1 g of yew extract was fractionated by HPLC with the LC I solvent system since this solvent was easier to evaporate. The substance had identical chromatographic properties as the substance identified in the stomach content with a retention time of 6.3 mins. The yield was about 5 mg of the unknown substance. This was analysed with IR and ^{1}H-NMR spectroscopy and the results are shown in Figs. 5 and 6.

By combining the measured spectroscopic data, the supposed structure of the unknown substance present in yew leaves as well as in stomach contents and cardiac



3,5–Dimethoxyphenol

Fig. 7. Structure of 3,5-dimethoxyphenol (phloroglucindimethylether)

blood of the victim could be definitely confirmed as phloroglucinedimethylether (3,5-dimethoxyphenol, Fig. 7). This could be confirmed by comparison with the pure substance purchased from Sigma (see Fig. 6).

To determine the recovery rates stomach contents of cases of natural death examined at the institute ($n = 5$)

were spiked with 20 mg/kg of 3,5-dimethoxyphenol. Five specimens of cardiac blood were spiked with 2.5 mg/kg. The recovery rates (mean ± SD) were 95.2% (± 2.3%) for stomach contents and 93.4% (± 3.5%) for cardiac blood. The day-to-day precision coefficients of variance (CV) were 4.8% for stomach contents and 5.1% for cardiac blood.

Quantification of 3,5-dimethoxyphenol was achieved using HPLC with the LC II solvent. The content in yew leaves harvested from the same yew tree was measured in January and in September. In January the content was 0.9 mg/g and in September 1.2 mg/g. The stomach contained 20 mg/kg and cardiac blood 0.32 mg/kg.

Thuja occidentalis L. – one member of another toxic plant genus was also examined using the described extraction procedure but 3,5-dimethoxyphenol was not detected in the leaves.

Lastly, a blood alcohol concentration of 1.31 g/kg was found.

Discussion

In this case of fatal intoxication by ingredients of Taxus baccata L. the first hints at the cause of the intoxication were derived from the case history and from the pharmacognostic aspect of the stomach and intestinal contents. Compared to other reports however toxicological proof of yew leaf ingestion and resorption of the ingredients could be presented.

In 1965 Frohne and Pribilla [11] reported a similar case, where they had characterized a substance both in harvested yew leaves, in leaves collected from the gastrointestinal tract, in the residual gastrointestinal fraction and in the liver. This substance was supposed to be taxin, which is now known to be a mixture of several alkaloids [13, 16, 17, 22, 26].

With the phloroglucindimethylether (3,5-dimethoxyphenol) detected here however, the aglycone of taxicatine [24, 25] was identified. Although none of the alkaloids were detected, both substances may serve as a indicators of an intoxication with yew ingredients, since especially 3,5-dimethoxyphenol is resorbed into the blood, as demonstrated in this case. Taxicatine and 3,5-dimethoxyphenol are considered to be yew specific ingredients [24, 25, 30] and 3,5-dimethoxyphenol can easily be derived from taxicatine by glucosidic cleavage.

3,5-dimethoxyphenol and several isomers have been reported in other sources such as tobacco [19, 20] and in pyrolytic lignin products [33]. These isomers had been determined by GC/MS, however a definite structure analysis such as the ^{1}H-NMR analysis used here, was not reported.

With the extraction procedure used we could demonstrate 2 Dragendorff positive spots with TLC, however data from the MS did not allow an identification.

Morphological and histological findings are in concordance with the cases reported so far [5, 11, 34]. The marked congestion of the inner organs of the victims indicate a subacute cardiocirculatory failure. Distinct cell lesions of myocytes and of Purkinje cells of the atrioventricular node might be the expression of a primary cardiac toxicity of the taxus alkaloids as observed in the hospitalized cases [2, 4, 6, 21, 34, 37], however these histological findings are not specific.

Reports [6, 34] indicate that fatal intoxications with yew leaves can occur in man at amounts of 50–100 g. In our case the stomach contents alone contained about 150 g leaves and nearly all the intestines contained yew leaves. The total amount of ingested leaves must therefore have been considerably higher. The total amount of leaves ingested could not be estimated using the 3,5-dimethoxyphenol content of blood or stomach contents, since the toxicokinetic properties are not known.

The substance 3,5-dimethoxyphenol might play an additional role in the general toxicity of taxus. There are no exact data on it's toxicity in man, however cytotoxic effects in E. coli, in Mycobacterium smegmatis [27] and in Chinese hamster ovary (CHO) cells [10] have been reported. However, the major toxic compounds are the taxine-derived alkaloids and their breakdown products. In the case reported here the blood alcohol level of 1.31 g/kg will surely have contributed to the fatal outcome and may have accelerated the process. However, this can only be speculated, since the exact time course of the intoxication could not be elucidated.

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