



A neolignan-type impurity arising from the peracid oxidation reaction of anethole in the surreptitious synthesis of 4-methoxyamphetamine (PMA)

Dieter Waumans, Bas Hermans, Noël Bruneel, Jan Tytgat*

Laboratory of Toxicology, Eduard van Evenstraat 4, 3000 Leuven, Belgium

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Abstract

The neolignan-type substance 2,4-dimethyl-3,5-bis(4'-methoxyphenyl) tetrahydrofuran is presented as a new forensic marker compound for the peracid oxidation of anethole. It is hypothesized that the formation of a stable intermediary carbocation in the hydrolysis reaction of anethole epoxide is not only responsible for the presence of 1,2-diols (and its esters) and 4-methoxyphenyl-2-propanone (PMP2P) but can also be the cause for the creation of this neolignan impurity due to interaction with anethole itself. Moreover, the applicability of this new forensic marker is demonstrated by its retrieval in clandestinely manufactured 4-methoxyamphetamine (PMA) preparations.

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1. Introduction

The prospecting for synthesis impurities in illicitly produced drugs is one of the mainstays of forensic chemistry. It is an established fact that so-called impurity profiles can aid in the elucidation of the followed synthetic route and can be adopted in gaining information regarding underground distribution networks [1,2]. Hitherto, the majority of published scientific articles dealing with the topic discuss illicitly produced amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA), in which the emphasis usually is on the Birch reduction [3], LiAlH_4 reduction of nitrostyrene derivatives [4], the Leuckart reaction and/or other reductive amination procedures [5]. A recent surge in the incidence of 4-methoxyamphetamine (PMA) on the underground drug market necessitated the study of the impurities concomitantly formed during the surreptitious

synthesis of this drug as well. Blachut et al. [6,7], Kirkbride et al. [8] and Coumbaros et al. [9] have recently contributed to the structural elucidation of Leuckart-specific PMA contaminants. Our research group has demonstrated that anethole, a major compound in (star) anise oil, can be used as precursor for 4-methoxyphenyl-2-propanone (PMP2P), which will yield PMA via the Leuckart reaction [10]. Furthermore, it was also specified how 4-methoxyphenol was formed as specific impurity during the peracid oxidation of anethole.

The current report describes the occurrence of an unexpected neolignan-type impurity, viz. 2,4-dimethyl-3,5-bis(4'-methoxyphenyl) tetrahydrofuran (**1**). This impurity is formed during the peroxidation reaction step of anethole and has been communicated in the past regarding contaminants found in the industrial peroxidation reaction of anethole [11]. This intriguing impurity displays structural similarities with neolignans occurring in nature, e.g. magnosalicin (and its diastereomers) (**2**) [12]. However, (**1**) has hitherto been unnoticed as potential forensic marker. We have examined its presence by conducting a series of experiments involving different reaction conditions, and have compared our laboratory

* Corresponding author Tel.: +32-1632-3402;

fax: +32-1632-3405.

E-mail address: jan.tytgat@pharm.kuleuven.ac.be (J. Tytgat).

results with impurity profiles obtained from seized PMA preparations.

2. Methods and techniques

2.1. Chemicals and reagents

All solvents used in this work were of analytical grade and purchased from Acros Organics (Geel, Belgium). Anise oil (China, derived from *Illicium verum*) was obtained from Taiga International NV (Breendonk-Puurs, Belgium). All other reagents had been acquired from Merck (Darmstadt, Germany).

2.2. Instrumentation

Sample analysis was effected with gas chromatography–mass spectrometry (GC/MS), viz. an Agilent 6890 Plus gas chromatograph equipped with an Agilent 5973N mass selective detector (MSD) and electronic pressure programming. Helium was used as a carrier gas at a constant linear flow rate of 1.0 mL/min; the column was a 30 m × 0.250 mm × 0.25 μm VF-5 MS factorFour capillary.

The mass spectrometer operated from 36 to 400 amu in electron impact (EI) mode with an ionization energy of 70 eV. A solvent delay of 4.00 min was applied. Two different oven temperature programs and injection modes were used. Reaction mixtures: 1 μL injection (split 1:50), oven programming: 50 °C (held for 1 min), 35 °C/min to 100 °C, 10 °C/min to 270 °C (held for 5 min). Seized sample screening: 1 μL injection (split 1:10), 50 °C (held for 1 min), 5 °C/min to 270 °C.

2.3. Synthesis procedures

2.3.1. Preparation of performic and peracetic acid

Performic acid was prepared by adding 6.8 g freezer-cold 30% hydrogen peroxide to 24.0 g formic acid (98–100%). This mixture is stirred for ca. 1 h before using it in further syntheses. Due to the instability of performic acid, the solution has to be prepared fresh for every experiment. A stock solution of peracetic acid was prepared by combining 288.0 g of 30% hydrogen peroxide and 4.0 g concentrated sulfuric acid with 100.0 g glacial acetic acid. The reaction mixture was stored for 5 days in a dark and well-ventilated place, after which it was ready for use [13].

2.3.2. Peracid oxidation of anethole

2.3.2.1. Peracid oxidation of anethole dissolved in acetone. A 250 mL round-bottomed flask was equipped with a magnetic stirbar and a thermometer, and charged with a solution of 6.0 g anise oil in 30 mL acetone. Performic acid solution was added at such a rate that the reaction mixture temperature did not exceed 38 °C. After addition of the whole

performic acid solution, the reaction was allowed to continue for ca 12 h. The reaction mixture was poured in its equal volume of cold distilled water (dH₂O) and extracted with 2 × 50 mL dichloromethane (DCM). The yellow organic phase was isolated and washed with 75 mL of dH₂O, after which the organic phase was dried over Na₂SO₄. After evaporation of the solvent under reduced pressure, an aromatically scented yellow oil weighing 8.1 g remained.

Peracetic acid: substituting performic acid for 25.5 g peracetic acid solution yielded 5.8 g of a yellow oil after a similar work-up.

For both reaction mixtures, 100 μL of the oil was dissolved in 5 mL of methanol for further GC/MS analysis.

2.3.2.2. Peracid oxidation of anethole dissolved in dichloromethane. A 250 mL round-bottomed flask was equipped with a magnetic stirbar and a thermometer, and charged with a solution of 6.0 g anise oil in 25 mL of DCM. Performic acid solution was added to the vigorously stirred reaction mixture at such a rate that the reaction mixture temperature did not exceed 38 °C. The reaction was allowed to continue another 12 h after addition of the final performic acid solution. Subsequently, the reaction mixture was carried over to a separation funnel and the organic layer isolated. The aqueous phase was extracted with 50 mL of DCM and thereupon discarded. The combined organic phases were washed with 3 × 50 mL of dH₂O, after which it was dried over Na₂SO₄. This yielded 5.8 g of a bordeaux red viscous oil

Peracetic acid: substituting performic acid for 25.5 g peracetic acid yielded 4.9 g of a yellow oil after a similar work-up.

For both reaction mixtures, 100 μL of the oil was dissolved in 5 mL of methanol for further GC/MS analysis.

2.4. Extraction method

An aliquot of a seized sample (100 mg for powder and capsule content, 75 mg for pulverized tablet) was dissolved in 5 mL of 0.1 M hydrochloric acid. The solution was extracted with 5 mL of DCM by mixing it thoroughly with a rotamix device for 20 min. The organic phase was isolated and dried over Na₂SO₄, which was rinsed with 1 mL of DCM. The solvent was evaporated under a beam of nitrogen gas and the residue reconstituted in 75 μL of MeOH for GC/MS analysis.

3. Results and discussion

3.1. 2,4-dimethyl-3,5-bis(4'-methoxyphenyl) tetrahydrofuran in the performic and peracetic acid oxidation reaction of anethole

The presence of **(1)** in four reaction mixtures has been evaluated: performic and peracetic acid have been chosen as

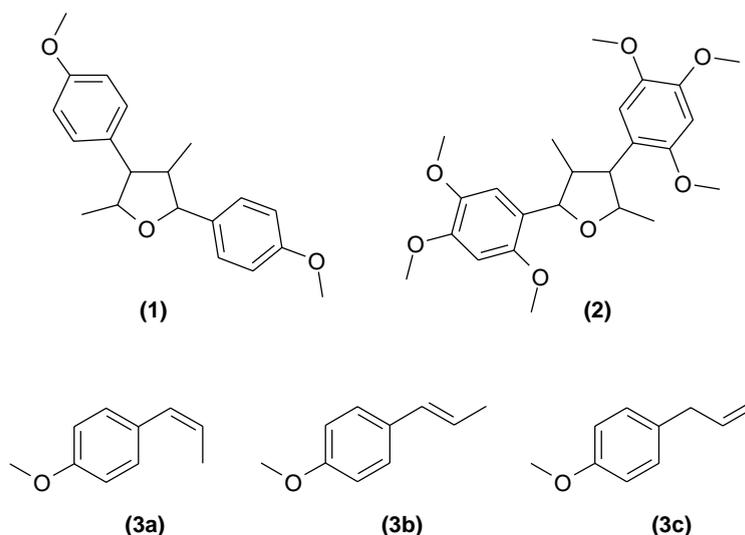


Fig. 1. Structural formulas: (1) 2,4-dimethyl-3,5-bis(4'-methoxyphenyl) tetrahydrofuran; (2) magnosalicin; (3a) *cis*-anethole; (3b) *trans*-anethole; (3c) methyl chavicol.

peracids, while acetone and DCM have been utilized as solvent. These are the most trivial choices when it comes down to simulating the peracid oxidation of a propenylbenzene in clandestine laboratories. Experiments conducted in the past revealed that anise oil had been used as PMA precursor, i.e. applied without prior purification of anethole by means of fractional distillation under reduced pressure. Hence, our choice for anise oil and not anethole. Moreover, it is known that anise oil has a very high anethole content [14]. As a rule, anise oil consists for 80–90% of anethole (*cis* and *trans* isomers, (3a) and (3b), respectively; predominantly present as *trans*) and there usually is a small percentage of methyl chavicol ((3c), the allyl isomer of anethole) as well (Fig. 1).

As can be seen in chromatograms [a] through [d] in Fig. 2, (1) is present in all four reaction mixtures after the described work-up. It was found that three chromatographic peaks have been retrieved for this neolignan, representing three out of four theoretically possible diastereomers for (1). Chromatogram [e] in Fig. 2 is the extracted ion chromatogram for m/z 268 from chromatogram [a] and indicates the presence of three diastereomers. Its mass spectrum is shown in the same figure together with the four possible configurations. Previous works discuss the NMR spectra of these compounds [11,15]. It is interesting to note, however, that this neolignan is also formed *in vitro* using performic acid and not alone with peracetic acid, as applied in these two references.

3.2. Investigation of the formation of 2,4-dimethyl-3,5-bis(4'-methoxyphenyl) tetrahydrofuran

The formation of this impurity is directly linked to the hydrolysis of anethole epoxide in the reaction mixture. As

shown in Fig. 2 and more detailed in Fig. 3, anethole is converted into its epoxide analogue by the applied peracid (in the present case, this would be performic or peracetic acid; Fig. 3 step A). This epoxide is hydrolyzed to its 1,2-diol analogue (step B) and is usually present as a formyl or acetyl derivative. By refluxing the 1,2-diol in a sulfuric acid-methanol mixture, it will be dehydrated to PMP2P (4) (step C, better known as the pinacol–pinacolone rearrangement). The latter can be used as precursor for PMA [6] (step G, by for instance the Leuckart reaction).

However, anethole epoxide can convert into the stable carbocation (5) as well (Fig. 3 step D). The latter can be reacted to its corresponding 1,2-diol with water (step F) or can be converted into (4) by hydrogen migration when (5) is present as a zwitterion (step E). The anethole epoxide derived presence of (5) has been described by Mohan et al. [16,17], who trapped it by means of forming the azide analogue. Interestingly, we found (4) in all described test runs (i.e. prior to the pinacol–pinacolone rearrangement!). This presence can now be explained via the intermediary formation of (5) as well.

The same carbocation (5) can be held responsible for the formation of (1). As shown in the reaction scheme in Fig. 2, anethole serves as precursor for both anethole epoxide (A)—and hence carbocation (5) (B)—as (1). Anethole and (5) will form an intermediary and unstable complex (C and D), which will be subject for an intramolecular rearrangement to yield (1) (E).

3.3. Screening for 2,4-dimethyl-3,5-bis(4'-methoxyphenyl) tetrahydrofuran in clandestine PMA preparations

A total of four different clandestine PMA preparations have been screened for the presence of (1). The

preparations have been confiscated by Belgian Law Enforcement in 2001/2002 and have been transferred to our laboratory for investigation. Previous findings have meanwhile entered the published scientific literature

[10]. The screened preparations include two brownish powders (seized from different sources), a capsule containing a similarly appearing brownish powder, and a beige tablet bearing an 'xTc'-logo. The latter has been

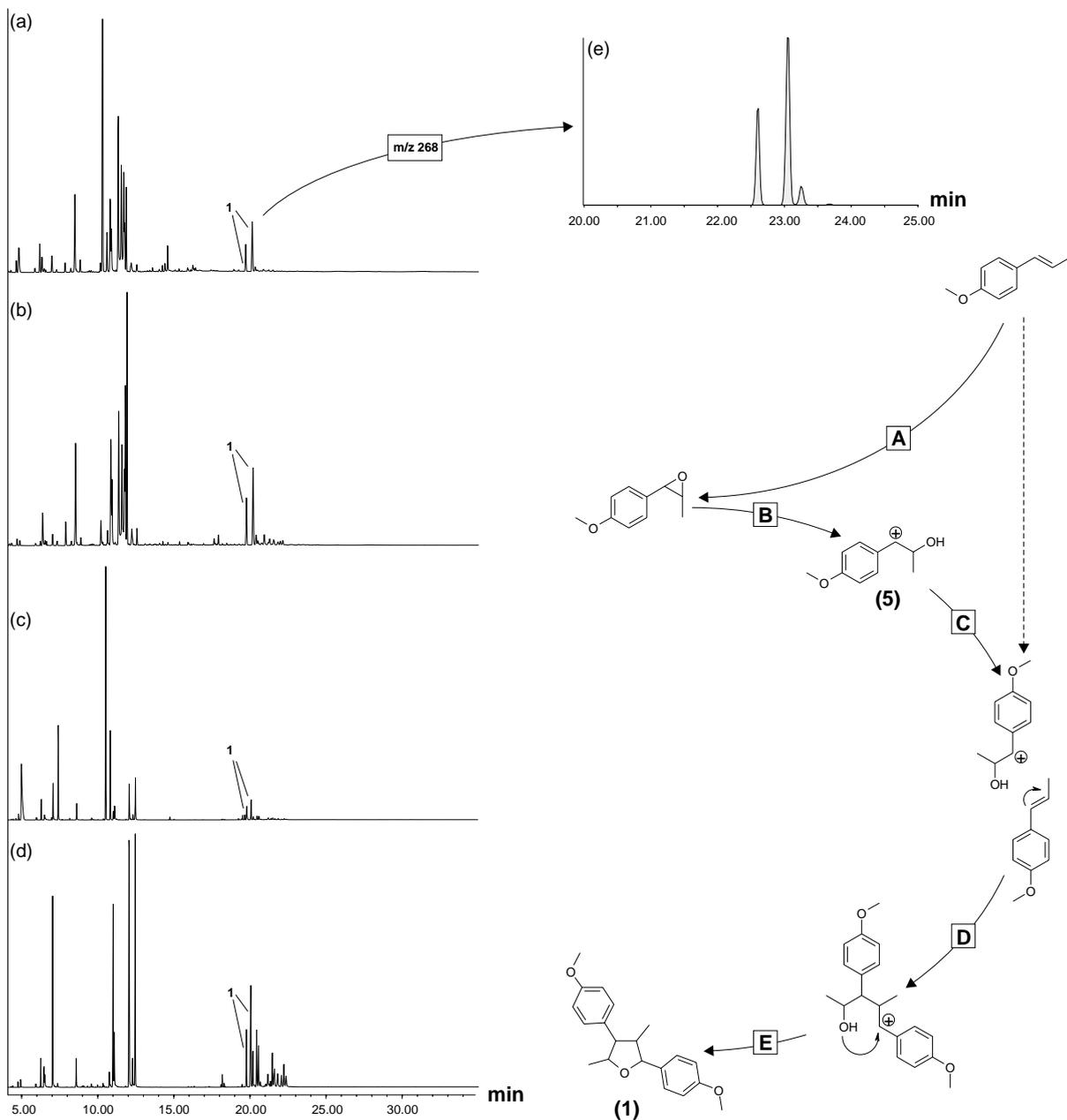


Fig. 2. Chromatograms: [a] performic acid oxidation in acetone [b] performic acid oxidation in DCM [c] peracetic acid oxidation in acetone [d] peracetic acid oxidation in DCM [e] extracted ion chromatogram (m/z 268) for retention time interval 20.00–25.00 from [a]. Mass spectrum: The diastereomers have identical mass spectra. The four theoretically possible diastereomers are shown. Reaction scheme: Anethole is epoxidized (A), after which the obtained anethole epoxide is converted to a stable carbocation (5) (B). Formation of neolignan (1) follows from the interaction between (5) and anethole (C), which results in the formation of an unstable intermediary product prone to intramolecular rearrangements (E).

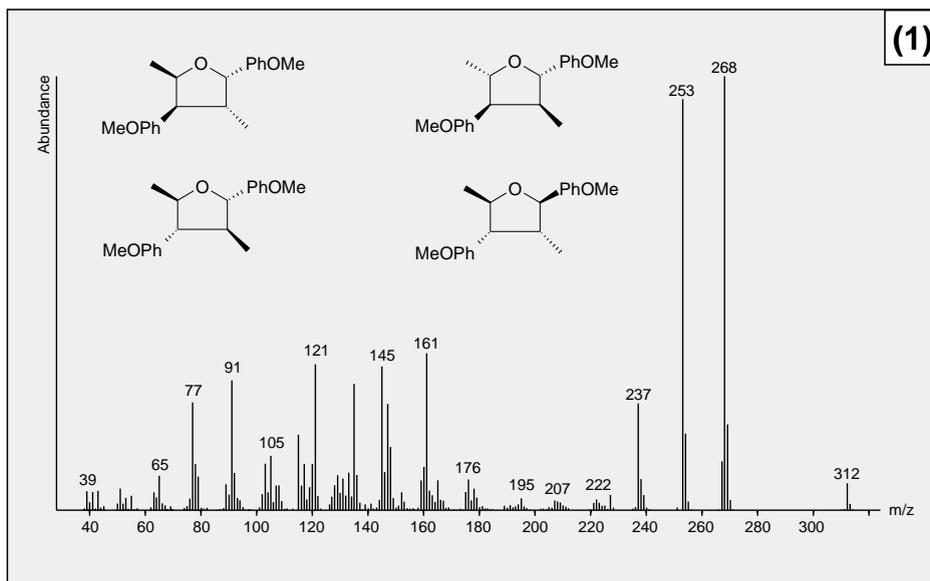


Fig. 2. (Continued).

communicated in the past to have been involved in at least two lethal accidents in Belgium [18].

The chromatographic results are shown in Fig. 4 and show the presence of **(1)** in all four samples, hereby indicating that they all have been manufactured in a similar way, viz. via peracid oxidation of anethole. Palmitic and stearic acid present in the tablet slightly interfere, but the extracted ion chromatogram for m/z 268 leaves little doubt (Fig. 4, chromatogram [e]). Furthermore, we found three

peaks for the different diastereomers in all screened samples; a similar finding has been reported earlier in this text for simulated experiments.

Other distinctly present and previously described impurities are 4-methoxyphenol [10] (**A**) as anethole peroxidation marker, and 4-methyl-5-(4'-methoxyphenyl) pyrimidine [8] (**C**) as Leuckart reaction marker. The retrieval of 4-methoxyphenyl-2-propanol (**B**) points at the reduction of (**4**), which can occur during the Leuckart reaction as well [9].

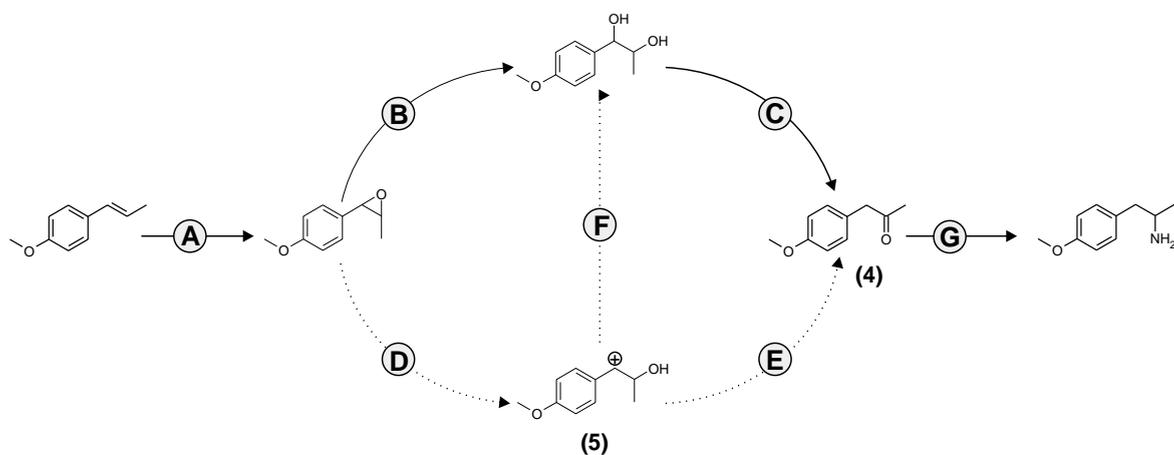


Fig. 3. Anethole is reacted to its epoxide analogue using performic or peracetic acid (**A**). This epoxide is hydrolyzed to the *vic*-diol derivative (**B**), which in turn is dehydrated in a sulfuric acid-methanol mixture in a next synthesis step to yield 4-methoxyphenyl-2-propanone (**4**) (**C**). Anethole epoxide can also be converted to an intermediary carbocation (**5**) (**D**), which can be reacted with water to yield the *vic*-diol (**F**) or it can rearrange to (**4**) in its zwitterionic form (**E**). (**4**) can be subjected to e.g. the Leuckart reaction to obtain 4-methoxyamphetamine (PMA).

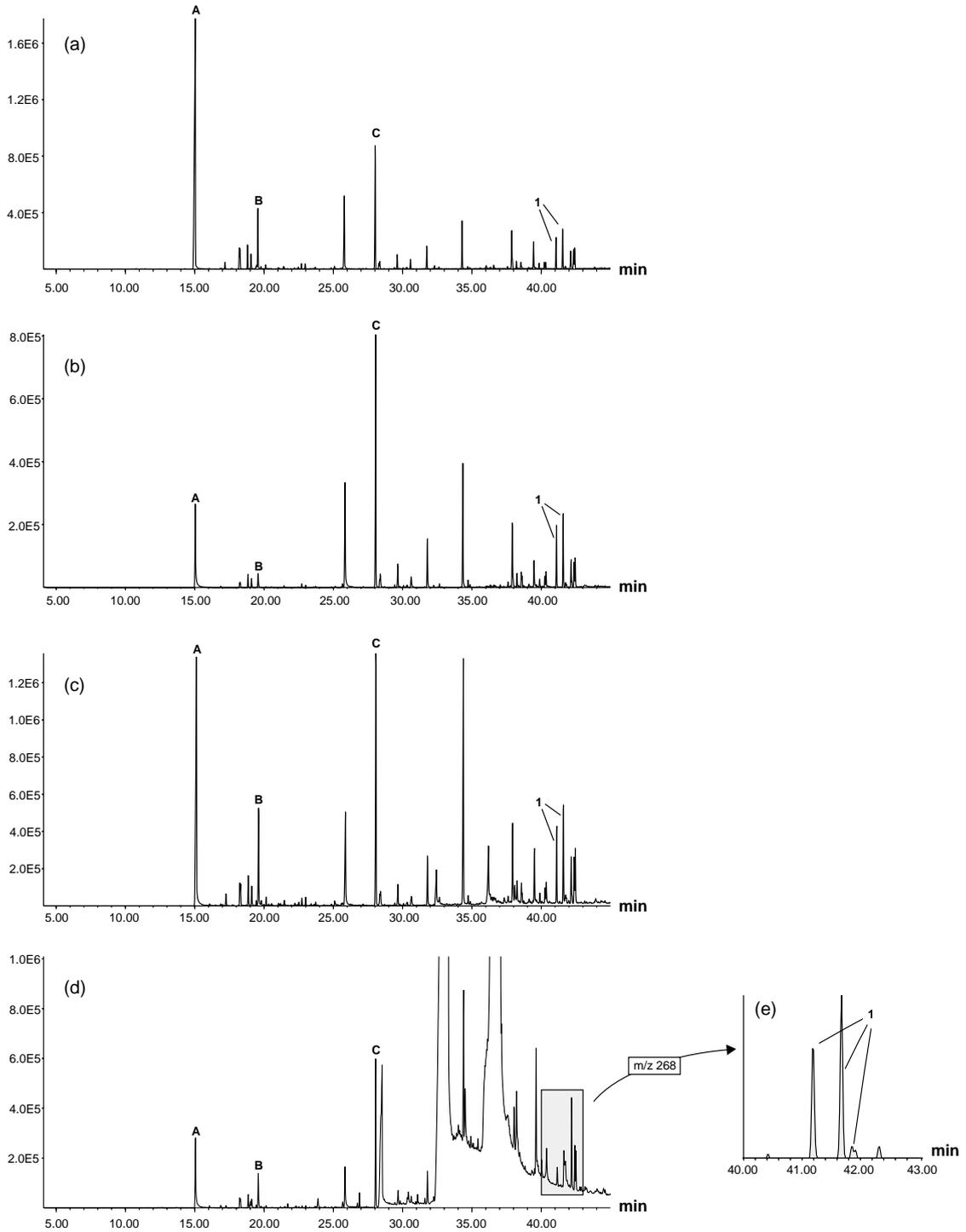


Fig. 4. Chromatograms: [a] and [b] are both brownish powders, [c] is a brownish powder contained in a capsule and [d] is a tablet extract. [e] is the extracted ion chromatogram for m/z 268 for retention time interval 40.00–43.00 for [d].

4. Conclusion

It has been found that 2,4-dimethyl-3,5-bis(4'-methoxyphenyl) tetrahydrofuran, a chemical substance with a neolignan structure, is formed during the performic and peracetic acid mediated oxidation of anethole. Taking into consideration the manner this impurity is formed during the reaction, it can be argued that this compound is a selective marker for the peracid oxidation reaction of anethole. Its applicability is demonstrated by its presence in clandestinely manufactured preparations. It should be noted, however, that the presence of this impurity depends on a great deal on the underground chemist's work-up abilities and/or mindset. Since the new impurity is a high-boiling substance, it is unlikely to retrieve it if intermediary purification of PMP2P has occurred.

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References

- [1] D.G. Sanger, I.J. Humpreys, A.C. Patel, M. Japp, R.G.L. Osborne, The significance of gas chromatographic impurity patterns obtained from illicitly produced amphetamine, *Forensic Sci. Int.* 28 (1985) 7–17.
- [2] W. Krawczyk, A. Parczewski, Application of chemometric methods in searching for illicit Leuckart amphetamine sources, *Anal. Chim. Acta* 446 (2001) 107–114.
- [3] K.L. Windahl, M.J. McTigue, J.R. Pearson, S.J. Pratt, J.E. Rowe, E.M. Sear, Investigation of the impurities found in methamphetamine synthesized from pseudoephedrine by reduction with hydroiodic acid and red phosphorus, *Forensic Sci. Int.* 76 (1995) 97–114.
- [4] J. DeRuiter, C.R. Clark, F.T. Noggle, Gas chromatographic and mass spectral analysis of amphetamine products synthesized from 1-phenyl-2-nitropropene, *J. Chromat. Sci.* 32 (1994) 511–519.
- [5] A.M.A. Verweij, Clandestine manufacture of 3,4-methylenedioxymethylamphetamine (MDMA) by low pressure reductive amination. A mass spectrometric study of some reaction mixtures, *Forensic Sci. Int.* 45 (1990) 91–96.
- [6] D. Blachut, K. Wojtasiewicz, Z. Czarnocki, Identification and synthesis of some contaminants present in 4-methoxyamphetamine (PMA) prepared by the Leuckart method, *Forensic Sci. Int.* 127 (2003) 45–62.
- [7] D. Blachut, J.K. Maurin, K. Wojtasiewicz, W. Starosta, Z. Czarnocki, (2S)-1-(4-methoxyphenyl)-N-[(1R)-2-(4-methoxyphenyl)-1-methylethyl]-2-propanamine in crude *p*-methoxyamphetamine (PMA) produced by the Leuckart method, *Z. Naturforsch.* 57b (2002) 593–597.
- [8] K.P. Kirkbride, A.D. Ward, N.F. Jenkins, G. Klass, J.C. Coumbaros, Synthesis of 4-methyl-5-arylpyrimidines and 4-arylpyrimidines: route specific markers for the Leuckardt preparation of amphetamine, 4-methoxyamphetamine, and 4-methylthioamphetamine, *Forensic Sci. Int.* 115 (2001) 53–67.
- [9] J.C. Coumbaros, K.P. Kirkbride, G. Klass, Application of solid-phase microextraction to the profiling of an illicit drug: manufacturing impurities in illicit 4-methoxyamphetamine, *J. Forensic Sci.* 44 (6) (1999) 1237–1242.
- [10] D. Waumans, N. Bruneel, J. Tytgat, Anise oil as para-methoxyamphetamine (PMA) precursor, *Forensic Sci. Int.* 133 (2003) 159–170.
- [11] H.-P. Schmauder, D. Gröger, D. Lohmann, H. Grüner, H. Foken, A. Zschunke, Über Nebenprodukte einer technischen Anetholoxidation, *Pharmazie* 34 (1979) 22–25.
- [12] M. De Fátima Da Silva, F.S.N. Taveira, J. Guiliieme Soares Maia, L.M. Conserva, M. Yoshida, O.R. Gottlieb, The natural occurrence of magnosalicin diastereomers, *Phytochemistry* 45 (7) (1997) 1527–1528.
- [13] F.P. Greenspan, The convenient preparation of per-acids, *J. Am. Chem. Soc.* 68 (1946) 907.
- [14] E. Guenther, The essential oils, vol. IV (pp. 563–570), vol. IV (pp. 634–645) and Vol. V (pp. 361–379). D. Van Nostrand Company, New York 1950.
- [15] K. Mori, M. Komatsu, M. Kido, K. Nakagawa, A simple biogenetic-type synthesis of magnosalicin, a new neolignan with antiallergy activity isolated from *Magnolia salicifolia*, *Tetrahedron* 42 (2) (1986) 523–528.
- [16] R.S. Mohan, D.L. Whalen, Acid-catalyzed hydrolysis of *cis*- and *trans*-anethole oxides: discrete carbocation intermediates and *syn/anti* hydration ratios, *J. Org. Chem.* 58 (1993) 2663–2669.
- [17] R.S. Mohan, K. Gavardinas, S. Kyere, D.L. Whalen, Spontaneous hydrolysis reactions of *cis*- and *trans*-*b*-methyl-4-methoxystyrene oxides (anethole oxides): buildup of *trans*-anethole oxide as an intermediate in the spontaneous reaction of *cis*-anethole oxide, *J. Org. Chem.* 65 (2000) 1407–1413.
- [18] S. Voorspoels, V. Coucke, P. Schepens, Paramethoxyamphetamine: first fatalities in Belgium, *Bull. Int. Assoc. Forensic Toxicol.* 31 (4) (2002) 12–13.