Journal of Pharmaceutical and Biomedical Analysis 157 (2018) 116–121

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Gas chromatographic-mass spectrometric characterization of thebaol, an opium constituent, and its structural analogs

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ARTICLE INFO

Article history:
Received 19 March 2018
Received in revised form 8 May 2018
Accepted 12 May 2018

Keywords:
Thebaol
Phenols
GC–MS
Derivatization
Trialkylsilylation
Perfluoroacylation
Alkoxycarbonylation

ABSTRACT

A GC–MS method is described for the characterization of thebaol, a component of opium poppy. The method includes preliminary sample derivatization to TMS, TBDMS, TFA, PFP and HFB substituted products. Fragmentation of resulting derivatives is unique under electron ionization, and proceeds via consecutive loss of two radicals that violate the "even-electron rule". Peaks of $[M\text{-}2CH_3]^{+\cdot}$ and $[M\text{-}C_4H_9\text{-}CH_3]^{+\cdot}$ ions show maximum intensities in the spectra of trimethyl- and *tert*-butyldimethylsilyl-thebaols. Elimination of perfluoroalkyl and methyl radicals from $M^{+\cdot}$ is characteristic for TFA, PFP and HFB thebaols. The same fragmentation peculiarity is characteristic for derivatives prepared from related natural compounds containing vicinal 2-methoxyphenol moieties. The unique fragmentation of trialkylsilyl and perfluoroacyl derivatives of thebaol can be successfully used for thebaol determination within complex mixtures. This is part 4 from the series "Analytical derivatives in mass spectrometry", parts 1, 2 and 3 see [1–3].

1. Introduction

Thebaol, a condensed three-aromatic ring representative of the phenanthrenoid family, is found in opium poppy [1,2] along with naturally occurring alkaloids such as thebaine, morphine, codeine, and oripavine. It has been used as thebaine marker, while thebaine itself is being tested as a heroin marker [3,4]. Acetyl-thebaol and its N-methyl-acetylamidoethyl analog have been utilized by P. Chen and co-authors [5] to distinguish the origin of heroin in urine collected from heroin addicts: from 'street' heroin or poppy seed ingestion; these acetyl-thebaols were produced via acetylation of samples.

In the present study, we tested alternative chemical modifications of thebaol prior to GC–MS analysis. Derivatization is particularly beneficial for phenolic compounds which otherwise tail badly on most chromatographic phases. In many cases, suitable derivatization makes possible a good GC separation of phenolic components of a mixture. Furthermore, derivatives may promote distinguishable EI fragmentation patterns, and undergo distinctive and regio-specific fragmentation processes practical for structure determination [6]. Therefore, mass spectra of derivatives can facilitate unequivocal identification of ortho, meta and para isomers.

The central goal of the present study was the development of a dependable GC–MS method for trace analysis of naturally occurring phenols by exploration of their derivatization products and the discovery of the most effective derivative with characteristic mass spectrometry properties. Moreover, acquisition of reliable reference data for potential indicators of botanical origin is important for forensic analysis and systematic addition of various derivatives of targeted co-constituents of narcotic substances, enhances the forensic utility of the NIST/NIH/EPA mass spectral library [7]. The paper focuses on fragmentation pathways of thebaol derivatives along with similar derivatives of other naturally occurring structural analogs of thebaol containing a single aromatic ring. This paper is a part 4 from the series "Analytical derivatives in mass spectrometry", parts 1, 2 and 3 see [8–10].

Abbrevations: BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide; EI, electron ionization; EPA, Environmental Protection Agency; GC–MS, gas chromatography – mass spectrometry; GCRI, gas chromatography retention index; HFB, heptafluorobutyryl; HFBA, heptafluorobutyric anhydride; MTBSTFA, N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide; NIH, National Institute of Health; NIST, National Institute of Standards and Technology; PFP, pentafluoropropionyl; PFPA, pentafluoropropionic anhydride; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetyl; TFAA, trifluoroacetic anhydride; TMS, trimethylsilyl.

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[1] Certain commercial materials and instruments are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the identified materials are necessarily the best available for the purpose.

https://doi.org/10.1016/j.jpba.2018.05.016
0731-7085/

2. Materials and methods[1]

2.1. Chemicals

Thebaol (I) was purchased from Cerilliant/National measurement institute, West Lindfield, New South Wales, Australia; other initial compounds guaiacol (II) and substituted guaiacols (III–XVI) were acquired from Sigma-Aldrich, St. Louis, MO, USA (Fig. 1).

All nine analytical reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA): (1) BSTFA with 1% trimethylchlorosilane, (2) MTBSTFA with 1% *tert*-butyldimethylchlorosilane, (3) TFAA, (4) PFPA, (5) HFBA, (6) methyl chloroformate, (7) ethyl chloroformate, (8) *n*-propyl chloroformate and (9) isopropyl chloroformate.

Anhydrous acetonitrile, "biotech. grade" pyridine, 0.01 mol/L hydrochloric acid solution, "certified reference material" grade chloroform and Premium quality water were also obtained from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Micro-synthesis

2.2.1. Silylation

Phenolic compounds were converted to TMS and TBDMS derivatives by treating 2% phenol solutions in acetonitrile with BSTFA or MTBSTFA for 3 h at room temperature, according to the existing protocol [11].

2.2.2. Acylation

35 μL of acyl anhydride (TFAA, PFPA of HFBA) was added to a solution of 0.2 mg a substrate in 100 μL of acetonitrile. The reaction was accomplished in one hour at 30 °C to 40 °C. The Solvent, reagent and resulting perfluoro-acid were evaporated, and the derivatization products were re-dissolved in acetonitrile for the analysis [11].

2.2.3. Reaction of thebaol with alkyl chloroformate

Chemical modification is carried out with the use of a procedure described in [12]. For the synthesis of methoxycarbonyl derivatives 170 μL solution containing 25 mmol/L aqueous hydrochloric acid, methanol and pyridine in a volume ratio 8:4:1 was added to 1 mg of a phenolic compound followed by a slow addition of 5 μL of methyl chloroformate during 90 s at 20 °C. The solution was vortexed for 5 s, and then 100 μL of chloroform containing 1% methyl chloroformate was added followed by further vortexing for 10 s. After 15 min an aliquot was taken from the chloroform layer and analyzed by GC–MS.

2.3. Instrumentation

EI mass spectra and GCRI were recorded on Agilent 5977A GC–MS system (Agilent Technologies, Santa Clara, CA, USA) at ionization energy 70 eV and ion source temperature 230 °C. Separation of components was achieved in a split injection mode with a split ratio 40:1 and helium gas flow of 1 mL min^{-1} on a fused silica capillary column (30 m, 0.25 mm i.d.; non-polar stationary phase: polymethylsiloxane + 5% phenyl groups) with programmed oven temperature from 60 °C to 270 °C at a rate of 10 °C min^{-1} and the injection port temperature 270 °C.

3. Results & discussion

In the course of this study three types of derivatives have been obtained: trialkylsilyl (TMS and TBDMS), perfluoroacyl (TFA, PFP and HFB) and alkoxycarbonyl (methoxy, ethoxy, *n*-propyloxy and isopropyloxy). GC–MS measurements were made for each chemical under study and its 9 chemical modification products, and their GC-RI and mass spectral data were recorded.

3.1. Trialkylsilyl derivatives

Thebaol was selected as a model to investigate the loss of two alkyl radicals from $M^{+\cdot}$ of *ortho* substituted methoxy-trimethylsilyloxy aromatic compounds. Fig. 2A and B depict mass spectra of TMS and TBDMS derivatives of thebaol. TBDMS thebaol loses methyl and *tert*-butyl radicals from the $M^{+\cdot}$ at the expense of TBDMS substituent. The loss of the larger t-butyl vs. the methyl radical from the trialkylsilyl group is preferred as expected. The resulting cations readily eliminate methyl from the methoxy group giving rise to the formation of ions $[M - 2CH_3]^{+\cdot}$ and $[M\text{-}C_4H_9\text{-}CH_3]^{+\cdot}$ (Scheme 1). Elimination of two methyl radicals is observed in the case of TMS-thebaol. The driving force of these processes is the formation of radical cations at 296 Da probably possessing a stable skeleton of phenanthro[3,4-d]-1,3,2-dioxasilacyclopentane – a structure with a high level of aromaticity.

This loss of two alkyl radicals appears to violate the 'even electron rule' [13–15], that is, odd-electron cations (such as molecular ions or fragments formed by rearrangements) may eliminate either a radical or an even-electron neutral species, but even-electron ions (such as protonated molecules or fragments formed by a single bond cleavage) will not usually lose a radical to form an odd-electron cation. However, this process appears universal for TMS and TBDMS derivatives of 2 methoxyphenols (II)-(XVI), and results in the formation of dominant peaks in the spectra. In fact, peaks of $[M\text{-}R\text{-}CH_3]^+$ ions probably having a structure of 2-dialkylsila-1,3-dioxaindane show maximum (base peak) intensities in the spectra of TMS and TBDMS derivatives of guaiacols (II–V, XIII–XIV), syringol (VI), 2,6- (VII) and 2,5-dimethoxyphenol (VIII), isovanillin (XI), vanillin (XII) and substituted benzenediol (XVI). The exceptions are tri- and tetra-trialkylsilyl derivatives of normetadrenaline (XV), and di-trialkylsilyl derivatives of isovanillyl (IX) and vanillyl alcohols (X).

While isovanillyl (IX) and vanillyl alcohols (X) with derivatized phenolic hydroxyl violate the 'even electron rule' and produce mostly $[M\text{-}R\text{-}CH_3]^{+\cdot}$ radical cations (Table 1), mass spectra of their di-TMS and di-TBDMS analogs show a dominant peak at 209 Da that is a result of a competing α –C—C bond cleavage, producing $[M\text{-}OSi(CH_3)_3]^+$ ions; the intensities of peaks of $[M\text{-}2CH_3]^+$ ions are in a range of 0.2% to 58%. The competing process of a C—C-bond cleavage located at β-position to both an aromatic ring and the amino function, becomes dominant in fragmentation of trialkylsilyl derivatives of normetadrenaline. As a result, the peaks of iminium and oxonium cations dominate in the spectra; the formation of $[M\text{-alkyl-}CH_3]^{+\cdot}$ ions are diminished (Table 1).

Consistent with the mechanism in Scheme 1, the loss of two radicals requires *ortho* location of oxygen atoms in an aromatic ring leading to an "ortho" effect [16]; for *meta* and *para* isomers no analogous ions are observed.

3.2. Perfluoroacyl derivatives

Similar *ortho* substituent effects are observed upon electron ionization in the case of TFA, PFP and HFB derivatives of thebaol. The mass spectrum of O-PFP-thebaol depicted in Fig. 2C demonstrates prominent peaks at 281 Da and 266 Da corresponding to ions $[M\text{-}C_nF_{2n+1}]^+$ and $[M\text{-}C_nF_{2n+1}\text{-}CH_3]^{+\cdot}$; the $M^{+\cdot}$ shows maximum intensity in the spectrum. Other significant peaks in the spectrum correspond to $[M\text{-}C_nF_{2n+1}CO]^+$ ion at 253 Da, and ions in the mass region 139 Da – 237 Da represent fragmentation of the aromatic skeleton.