Research Institute for Medical & Health Sciences[1], University of Sharjah, Sharjah, United Arab Emirates; College of Medicine and Health Sciences[2], Khalifa University, Abu Dhabi, United Arab Emirates; Herbert Wertheim College of Medicine[3], Florida International University, Miami, USA

Possible metabolic conversion of pinene to ionone

T. H. Al-Tel[1], H. Tarazi[1], L. O. Aloum[2], D. E. Lorke[2,3], G. A. Petroianu[2,3,*]

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**Corresponding author: Georg A. Petroianu MD, PhD, College of Medicine and Health Sciences, Khalifa University, Department of Pharmacology & Therapeutics, PO Box 12 77 88, Abu Dhabi, United Arab Emirates georg.petroianu@ku.ac.ae*

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The unintended consequence of the ingestion of certain foods to alter the scent or color of urine is well known. Less awareness exists regarding the practice of ingestion of natural products or drugs with the intended purpose of conferring urine the scent of violets. The resin of the terebinth tree and the derived turpentine were widely used in antiquity in wine-making, both as taste enhancer and conserving agent, so the effect on urine was possibly noticed due to the presence in wines. It is also possible that turpentine's effect on urine was noticed subsequent to its use as medicine, as a component of various remedies popular in those days. The scent altering effect requires metabolic conversion of pinene, the main turpentine component to ionone, the molecule mainly responsible for the scent of violets. The metabolic pathway (in humans or otherwise) was (to our knowledge) not yet described. We here propose a possible metabolic pathway for the conversion of pinene to ionone, explaining the scent altering effect of turpentine. We also provide calculated pharmacokinetic (pK) data for the mentioned substances.

1. Introduction

The unintended consequence of the ingestion of certain foods of drastically altering the scent (asparagus) or color (beetroot) of urine is well known (Mitchell 2001). Less awareness exists regarding the ingestion of food or natural substances with the intended purpose of conferring urine a more pleasant scent: the resin of the terebinth tree (*Pistacia terebinthus*) and the derived distillate (*Oleum terebinthinae*), juniper berries (*Fructus juniperi*) and the oil (*Oleum juniperi*) as well as eucalyptus oil, all can to various degree confer urine a scent reminiscent of violets (Petroianu et al. 2018).

The most efficacious – according to lore – appears to be the distillate from the *terebinth* resin: the oil of turpentine. Turpentine ingestion for this specific purpose is attributed to Queen Cleopatra (French 1964; Apley 1973) and later also to Roman demi-monde belles (Heine 1844; Rutgers 1922; Rivière 2005).

Turpentine's effect on urine was initially noticed subsequent to its use either as medicine or to its use in wine-making, both as taste enhancer and conserving agent. As a consequence there was widespread exposure of the population to and awareness of its effects (Weber 1826; McGovern 2003; Norton 2006). The scent of violets is due to various mono-cyclical terpenoids, mainly ionones (C_{13}) and irones (C_{14}). Ionone synthesis was first achieved in 1893 (Tiemann and Krüger 1893; Petroianu et al. 2018; Petroianu 2019). The ability not only of ingested, but also of topically applied or inhaled turpentine essence to confer a violet-like scent to urine was experimentally confirmed by Sloane (1743) and later by Stehberger (1827). The fate of turpentine oil in the human body was examined by Friedrich Wöhler (1800–1882). He pointed out that internal and external use of this oil, as well as the inhalation of its vapor, quickly communicate a violet odor to the urine. Mixing oil of turpentine with urine however does not generate the violet smell (Woehler 1824). The topic was also examined by Edmund Sachs (1837–1907): he noted that only oil of turpentine but not colophony (rosin) produces the violet scent in urine in humans. Furthermore he pointed out that admixture of turpentine (oil of) to urine does not create the violet scent either (Sachs 1862). This indicates the need for metabolic conversion of turpentine oil components in order to obtain the violet-like scent. The metabolic pathway (in humans or otherwise) was (to our knowledge) not yet described (Vespermann et al. 2017).

Theodor Brümmer (1873–1959) examined the effect of daily administration of a low dose of oil of turpentine in humans: when receiving daily 20 drops (1 mL) of a 0.1% oil of turpentine solution the violet scent of urine appears after 3-4 days, or after administration of a cumulative dose of 4 mg of oil of turpentine (Brümmer 1900).

The most prevalent components in (the oil of) turpentine are the bicyclic mono-terpenes (C_{10}) α - and β -pinene, with lesser amounts of carene, camphene and limonene, and trace amounts of many other compounds (Table 1).

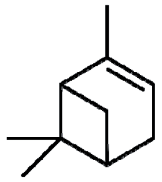
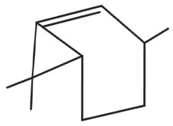
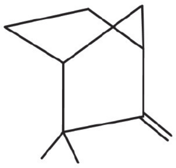
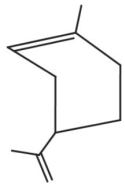
After exposure to pinene, less than 0.001% of the total uptake was eliminated unchanged in the urine and about 8% in exhaled air. The high clearance of α-pinene indicates that α-pinene is readily metabolized (Falk et al. 1990).

Falk Filipson confirms these findings and provides additional pK data. The mean relative uptakes of α-pinene, β-pinene, and 3-carene were 62%, 66%, and 68% respectively, of the amount supplied (inhalation) (Falk Filipson 1996).

These results are in-line with the experimentally determined logP (lipophilicity) of the turpentine components: 4.44 (Griffin et al. 1999) and 4.83 (Li and Perdue 1995) for α-pinene, 4.16 (Griffin et al. 1999) for β-pinene, 4.38 carene, 4,22 camphene (Griffin et al. 1999) and 4.57 limonene (Li and Perdue 1995).

The monoterpenes were readily metabolised and only small percentages of the total uptake were eliminated as unchanged monoterpene through the lungs (between 2% and 5% of the net uptake) and even less in the urine. The mean blood clearance 21 h after exposure of α-pinene, β-pinene and 3-carene, were 0.8, 0.5, and 0.4 l.kg-1.h-1, respectively. Conversion of pinene to ionone and/or ionone-like compounds is expected to be accompanied by a reduction in logP (increase in hydrophilicity). Griffin et al. (1999) determined experimentally logP for α-ionone as 3.85. Many other sources quote for ionone values between 2.9 and 3.9. The reduc-

Table 1: The main components in the oil of turpentine. Pinene is the most prevalent constituent while carene, camphene and limonene are present in lesser amounts.

Pinene	
Carene	
Camphene	
Limonene	

tion in logP favors subsequent renal elimination as evidenced by the documented violet-like scent of urine after turpentine exposure (Petroianu et al. 2018).

Chithalen et al. (2002) noted that cytochromes P450 (CYP) hydroxylation of β-ionone is highly efficient and that there is little accumulation of intermediates unless the substrate concentration is raised into the low micromolar range (as would be the case with intentional or accidental ingestion). Rabbits dosed orally with β-ionone excreted in the urine unchanged β-ionone (Ide and Toki 1970). Ionone induced CYP1A and CYP2B; induction by β-ionone was higher than that by α-ionone in both the mRNA and protein levels (Jeong et al. 2002).

The acute toxicity of α-ionone, β-ionone and ionone standard (60% α-ionone, 40% β-ionone) is low (Lalko et al. 2007; Belsito et al. 2007).

2. Investigations, results and discussion

2.1. Possible metabolic conversion of pinene to ionone

We propose a possible metabolic pathway that might lead to α-and β-ionone based on the enzymatic oxidation of α-pinene and β-pinene, respectively (Fig. 1: Pathway 1a & 1b) (Hampel et al. 2007; Krings et al. 2009; Negoi et al. 2018). Oxidation of α-pinene or β-pinene by CYP might lead to intermediates **I** and **Ia**, respectively. Rearrangement of the free radicals in **I** and **Ia**, should deliver intermediates **II** and **IIa**, respectively. Then, intermediate **IIa** should abstract an $H^•$ from CYP to deliver intermediate **III**a. The latter, will lose the allylic $H^•$ to CYP to produce intermediate **I**. At this stage, the two routes converge through the formation of intermediate **II**. The latter is formed through free radical rearrangement and cleavage of the cyclobutyl ring leading to **II**. Subsequent oxidation of, intermediate **II** should be oxidized to furnish the alcohol **III** which upon oxidation produces the aldehyde **IV**. At this junction, Knoevenagel condensation of **IV** with acetone should produce intermediate **V**. 1,5-sigmatropic rearrangement of **V** should produce intermediate **VI.** Rearrangement of the highly conjugated system in **VI**

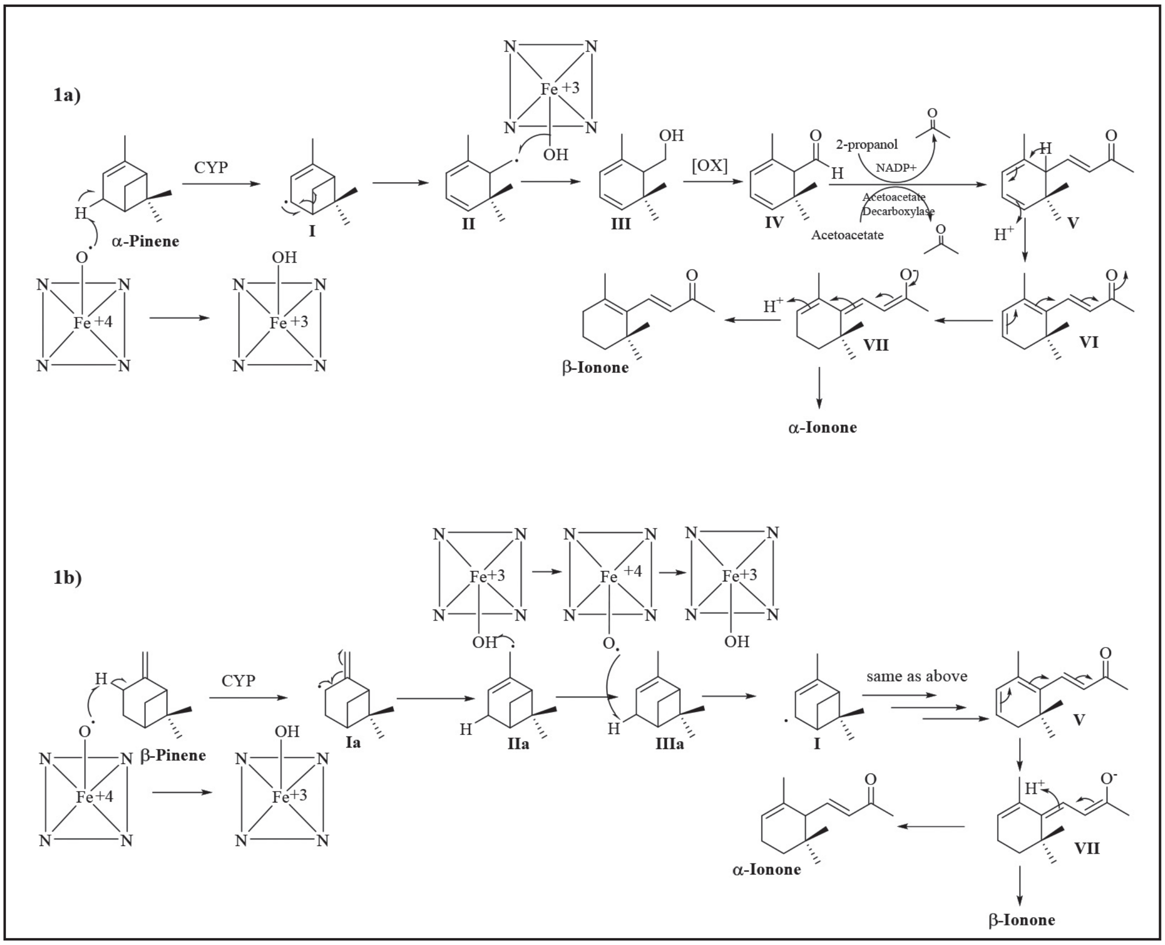


Fig. 1: Possible metabolic pathways to α-and β-ionone based on the enzymatic oxidation of α-pinene and β-pinene.

Table 2: Physicochemical and ADME properties for the compounds of interest

Property/Structure	α-Pinene	β-Pinene	(±)-Carene	(±)-Camphene	(±)-Limonene	α-Ionone	β-Ionone
TPSA[a] (Å^2)	0.00	0.00	0.00	0.00	0.00	17.07	17.07 Å
XLOGP3[b]	4.48	4.16	4.38	4.22	4.57	3.85	2.91
MLOGP[c]	4.29	4.29	4.29	4.29	3.27	2.94	2.94
Caco-2[d] (cm/s)	-4.33	-4.33	-4.30	-4.33	-4.33	-4.33	-4.31
Pgp-inhibitor	–	–	–	–	–	–	–
Pgp-substrate	–	–	–	–	–	–	–
HIA[e]	++	++	++	++	++	++	++
F (30%)	+	+	+	++	+	+	+
PPB (%)	68.41	67.00	68.61	69.10	75.30	81.74	81.42
VD[f] (L/Kg)	0.58	0.51	0.53	0.46	0.39	0.38	0.38
BBB	+++	+++	+++	+++	+++	+++	+++
$T_{1/2}$[g] (hrs)	1.88	1.79	1.94	1.83	1.74	1.62	1.63
CL[h] (mL/min/kg)	1.81	1.85	1.81	1.87	1.69	1.80	1.78
hERG-block activity	–	–	–	–	–	–	–
LD50[i] (mg/kg)	3778.3	4258.9	4308.2	4048.5	4789.6	3531.7	3597.4

a: Topological polar surface area calculated as per Ertl et al. (2000).
b: Log $P_{o/w}$ atomistic and knowledge based method calculated by XLOGP program, version 3.2.2.
c: Log $P_{o/w}$ topological method calculated according to Moriguchi et al. (1994).
d: Optimal: -4.70 to -4.80
e: Human intestinal absorption, where ++ ≥30%
f: Volume of distribution
g: Elimination half-life
h: Clearance
i: Acute toxicity

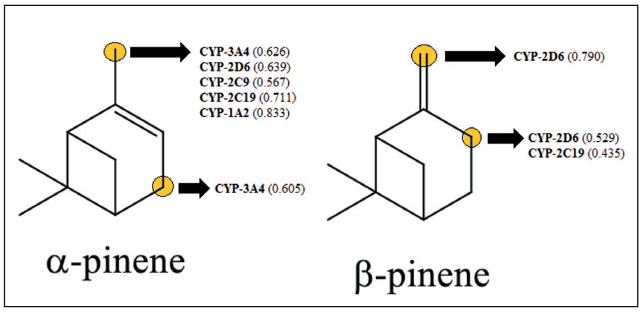


Fig. 2: Predicted site of metabolism (SOM) for the pinene monoterpenes along with the cytochrome P-450 isoforms involved, where, values between brackets indicate the probability of involvement.

should deliver the trienol **VII.** The latter can easily rearranges to both α-and β-ionone as shown in pathways 1a and 1b. The two routes might converge as a result of 1,5-sigmatropic rearrangement of **V** leading to intermediate **VI**. One of the key component of this proposed metabolic transformation is acetone. Acetone could be produced in organisms either by the decarboxylation of acetoacetate or the dehydrogenation of propan-2-ol according to the proposed routes in pathways 1a and 1b. It is well documented that acetoacetate decarboxylation is the major source of acetone in mammals, which could arise from either lipolysis or degradation of amino acid (Kalapos 2003; Koorevaar and Van Stekelenburg 1976; Nordmann et al. 1973; Theorell et al. 1951).

2.2. Computational details

The physicochemical parameters (TPSA, XLOGP3 and MLOGP) were calculated employing the SwissADME webserver (Daina et al. 2017). The parameters related to absorption, distribution, elimination and toxicity were calculated using the ADMETLab webserver (Dong et al. 2018). Additionally, *in-silico* prediction for the site of metabolism and the human CYP involvement were achieved *via* the SOMP webserver (Rudik et al. 2015).

According to the calculated data given in Table 2, the physicochemical parameters of α and β-pinene demonstrate their lipophilic nature. Their membrane permeation is significant and favors intestinal absorption and blood bioavailability of more than 30%. Their computed Volume of Distribution (Vd) indicates an even tissue distribution and the ability of reaching the site of metabolism as well as the ability to penetrate the blood brain barrier.

Toxicity related parameters such as hERG-blocking activity and LD50 indicated their safety profile. α- and β-ionones showed higher hydrophilicity as indicated by their computed PSA and Log $P_{o/w}$ which are consistent with the previously measured experimental values and favor renal elimination.

The predicted sites of metabolism (SOM) of the pinene monoterpenes with respect to the corresponding human CYP isoform involvement are given in Fig. 2. The predicted sites are consistent with the known metabolic reactions especially; allylic oxidation, epoxidation and stereoselective *gem*-dimethyl hydroxylation (Ishida et al. 1981; Eriksson and Levin 1996). Furthermore, the predicted CYP isoforms with high probabilities were; 1A2, 3A4, 2C19 and 2D6. Recently, an X-ray crystal structure of α-pinene with CYP 2B was resolved (PDB-ID: 4I91) confirming the role of CYP 2B6 and 2A6 in their metabolism (Wilderman et al. 2013; Shah et al. 2015).

2.3. Conclusion

Terebinth resin and oil were widely used in antiquity both for medicinal and wine preservation purposes. The effect of ingestion on the scent of urine could not have gone unnoticed. A metabolic pathway for the synthesis of α and β-ionone (responsible for the violet scent) from α and β-pinene (main components in turpentine) is proposed, explaining the scent altering effect of turpentine exposure. PK data for the compounds of interest is provided.

Conflict of interest: None declared.

References

Apley J (1973) Urine Examination. In: Paediatrics. Bailliere Tindall, p. 265.

Belsito D, Bickers D, Bruze M, Calow P, Greim H, Hanifin JM, Rogers AE, Saurat JH, Sipes IG, Tagami H (2007) A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients. Food Chem Toxicol 45: S130–S167.

Brümmer T (1900) Wie wirkt das Terpentinöl in kleinen Dosen längere Zeit genommen? MD thesis, University of Greifswald.

Chithalen JV, Luu L, Petkovich M Jones G (2002) HPLC-MS/MS analysis of the products generated from all-trans-retinoic acid using recombinant human CYP26A. J Lipid Res 43: 1133–1142.

Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7: 42717.

Dong J, Wang NN, Yao ZJ, Zhang L, Cheng Y, Ouyang D, Lu AP, Cao DS (2018) ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. J Cheminform 10:29. http://admet.scbdd.com/home/index/

Eriksson K, Levin JO (1996) Gas chromatographic-mass spectrometric identification of metabolites from alpha-pinene in human urine after occupational exposure to sawing fumes. J Chromatogr B Biomed Appl 677: 85–98.

Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 43: 3714–3717.

Falk AA, Hagberg MT, Löf AE, Wigaeus-Hjelm EM, Wang ZP (1990) Uptake, distribution and elimination of alpha-pinene in man after exposure by inhalation. Scand J Work Environ Health 16: 372–378.

Falk Filipson A (1996) Short term inhalation exposure to turpentine: toxicokinetics and acute effects in men. Occup Environ Med 53: 100–105

French EB (1964) Alimentary and Genito-Urinary System. In: Macleod JG: Clinical Examination: A textbook for students and doctors by teachers of the Edinburgh Medical School. E & S Livingstone, Edinburgh & London, p. 247.

Griffin S, Wyllie SG, Markham J (1999) Determination of octanol-water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography. J Chromatogr A 864: 221–228.

Hampel D, Swatski A, Mosandl A, Wüst M (2007): Biosynthesis of monoterpenes and norisoprenoids in raspberry fruits (Rubus idaeus L.): The role of cytosolic mevalonate and plastidial methylerythritol phosphate pathway. J Agric Food Chem 55: 9296–9304.

Heine H (1844) Deutschland. Ein Wintermärchen. Neue Gedichte, Hamburg, p. 329.

Ide H, Toki S (1970) Metabolism of beta-ionone. Isolation, characterization and identification of the metabolites in the urine of rabbits. Biochem J 119: 281–287.

Ishida T, Asakawa Y, Takemoto T, Aratani T (1981) Terpenoids biotransformation in mammals III: Biotransformation of alpha-pinene, beta-pinene, pinane, 3-carene, carane, myrcene, and p-cymene in rabbits. J Pharm Sci 70: 406–415.

Jeong HG, Chun YJ, Yun CH, Moon CK, Lee HS, Han SS, Lee ES, Jeong TC (2002): Induction of cytochrome P450 1A and 2B by alpha- and beta-ionone in Sprague Dawley rats.Arch Pharm Res 25: 197–201.

Kalapos MP (2003) On the mammalian acetone metabolism: from chemistry to clinical implications. Biochim Biophys Acta 1621: 122–139.

Koorevaar G, Van Stekelenburg GJ (1976) Mammalian acetoacetate decarboxylase activity. Its distribution in subfractions of human albumin and occurrence in various tissues of the rat. Clin Chim Acta 71: 173–183.

Krings U, Lehnert N, Fraatz M A, Hardebusch B, Zorn H, Berger RG (2009) Autoxidation versus biotransformation of r-pinene to flavors with Pleurotus sapidus: Regioselective hydroperoxidation of pinene and stereoselective dehydrogenation of verbenol. J Agric Food Chem 57: 9944–9950.

Lalko L, Lapczynski A, McGinty D, Bhatia S, Letizia CS, Api AM (2007) Fragrance material review on ionone. Food Chem Toxicol 45: S251–S257.

Li J, Perdue EM (1995) Physicochemical properties of selected monoterpenes. Pre-print Extended Abstract, Presented Before The Division of Environmental Chemistry, Amer Chem Soc, Anaheim, Ca: April 2-7

McGovern PE (2003) Ancient wine: the search for the origins of viticulture. Princeton University Press. Princeton & Oxford.

Mitchell SC (2001) Food idiosyncrasies: beetroot and asparagus. Drug Metabol Dispos 29: 539–543.

Moriguchi I, Hirono S, Nakagome I, Hirano H (1994) Comparison of reliability of log P values for drugs calculated by several methods. Chem Pharm Bull 42: 976–978.

Negoi A, Parvulescu VI, Tudorache M (2018) Peroxidase-based biocatalysis in a two-phase system for allylicoxidation of pinene. Catalysis Today 306: 199–206.

Nordmann R, Ribiere C, Rouach H, Beauge F, Giudicelli Y, Nordmann J (1973) Metabolic pathways involved in the oxidation of isopropanol into acetone by the intact rat. Life Sci 13: 919–932.

Norton S (2006) The pharmacology of Mithridatum: a 2000 year old remedy. Mol Intervent 6: 60–66.

Petroianu A, Stegmeier-Petroianu A, Lorke DE (2018) Cleopatra: from turpentine and juniper to ionone and irone. Pharmazie 73: 676–680.

Petroianu GA (2019) Veilchenduft, ectopic olfactory receptors and endogenous volatile compounds: Who was Paul Krüger (1859-1916)? Pharmazie 74: 443–446.

Rivière L (2005) Six hundred miseries: the seventeenth century womb. Book XV of the *Practice of physick*; Translated by Nicholas Culpeper in 1678. Edited and annotated by Burton John Lloyd. Royal College of Obstetricians and Gynaecologists Press, London

Rudik A, Dmitriev A, Lagunin A, Filimonov D, Poroikov V (2015) SOMP: web server for in silico prediction of sites of metabolism for drug-like compounds. Bioinformatics 31: 2046–2048 http://www.way2drug.com/SOMP.

Rutgers J (1923): The Sexual Life. In Its Biological Significance as a Dominant Factor of Vitality in Man and Woman, and in Plants and Animals Translated by Norman Haire (1892 – 1952). Verlag Richard A Giesecke; Dresden.

Sachs E (1862): Beiträge zur der Lehre vom Uebergange der Harze in das Blut: MD thesis, University of Dorpat

Shah MB, Wilderman PR, Liu J, Jang HH, Zhang Q, Stout CD, Halpert JR (2015):

Structural and biophysical characterization of human cytochromes P450 2B6 and 2A6 bound to volatile hydrocarbons: analysis and comparison. Mol Pharmacol 87: 649–659.

Sloane H (1743) Observations sur des odeurs communiquées au corps animal (Observations sur l'odeur de violette communiquée à l'urine, en mettant de la résine de pin sur la langue). Mémoires de l'Académie royale des sciences (Paris) 96-97

Stehberger GA (1827) Versuche über die Zeit, binnen welcher verschiedene in den menschlichen Körper aufgenommene Substanzen in dem Urin vorkommen. In: Tiedemann F, Trebiranus GR, Trebiranus LC (eds): Untersuchungen ueber die Natur des Menschen, der Tiere und der Pflanzen. Darmstadt, Leske CW, pp. 47–61.

Tiemann F, Kruger P (1893) Ueber Veilchenaroma. Ber Dt Chem Ges 26: 2675–2708.

Theorell H, Bonnichsen R (1951) Studies on liver alcohol dehydrogenase I. Equilibria and initial reaction velocities. Acta Chem Scand 5: 1105–1126.

Vespermann KAC, Paulino BN, Barcelos MCS, Pessôa MG, Glaucia M. Pastore GM, Molina G (2017) Biotransformation of α- and β-pinene into flavor compounds. Appl Microbiol Biotechnol 101: 1805–1817.

Weber W E (1826) Andromachus von Kreta. In: Die elegischen Dichter der Hellenen: Nach ihren Ueberresten übersetzt und erlaeutert. Verlag der Hermannschen Buchhandlung, Frankfurt am Main, pp. 764–767.

Wilderman PR, Shah MB, Jang HH, Stout CD, Halpert JR (2013) Structural and thermodynamic basis of (+)-α-pinene binding to human cytochrome P450 2B6. J Am Chem Soc 135: 10433–10440.

Wöhler F (1824) Versuche über den Übergang von Materien in den Harn. Zschr Physiol 1: 125–146; 290–317.