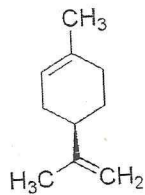


Limonen aus Orangenschalen



(R)-(+)-Limonen
((R)-1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene)

4 Orangen werden geschält, die Schalen in sehr kleine Stücke geschnitten, mit 500 mL dest. Wasser in den Dreihalskolben einer Wasserdampfdestillations-apparatur gegeben und mit einem Heizpilz zum Sieden erhitzt. Die Kühlung wird eingeschaltet und der Wasserdampf 2 h abdestilliert. Das Destillat wird zwei Mal mit je 40 mL Dichlormethan extrahiert, die vereinigten organischen Phasen über Magnesiumsulfat getrocknet, filtriert und am Rotationsverdampfer vom Lösungsmittel befreit.^[1]

Zur Charakterisierung wird ein IR-Spektrum gemessen und der Brechungsindex bestimmt.

Brechungsindex: 1.4730^[2]

Literatur: [1] C. Glidewell, *J. Chem. Educ.* **1991**, 68, 267-269, [2] R. C. Weast, M. J. Astle, *CRC Handbook of Chemistry and Physics*, 63. Auflage, CRC-Press, Boca Raton **1982**, C-361.

Monoterpenes

An Easily Accessible But Neglected Class of Natural Products

Christopher Glidewell

University of St Andrews, St Andrews, Fife KY16 9ST, UK

Plants have long been recognized as a major source of an extremely wide range of organic chemicals. Many compounds of plant origin are used in food flavorings and colorings, in perfumery, and as pharmaceuticals: this last group provides much of the basis for traditional and folk medicine.

One of the simplest groups of natural products of plant origin is the monoterpenes. As well as being structurally and spectroscopically straightforward, many of this group are key components of familiar flavors and odors: a number of them are also readily accessible experimentally, since they are volatile in steam and hence can be fairly easily extracted from fruits and seeds.

Despite their direct connection with everyday life, the study of the simple terpenes has almost disappeared from the normal undergraduate chemistry curriculum. This is a great pity since, in addition, the monoterpenes provide one of the simplest examples of a biosynthetic pathway, in a scheme that exemplifies straightforward applications of carbocation reactions, hydride shifts, and (*R, S*) stereochemistry that should be readily accessible to students in the early years of a chemistry degree program. This experience can then provide the foundation for the study of more elaborate areas of biological chemistry.

The biosynthesis of the monoterpenes proceeds from acetate units, in the form of acetyl coenzyme-A CH_3COSCoA , initially along the same mevalonic acid pathway that gives rise to the steroids (1, 2). The key intermediate for entry to the monoterpenes is neryl pyrophosphate (2), (Fig. 1), the geometric isomer of geranyl pyrophosphate (1), which is the point of divergence of monoterpene biosynthesis from that of higher terpenes and of steroids. This pyrophosphate (2) is a masked form of the carbocation (3), whose subsequent reactions can be used to rationalize the formation of a wide

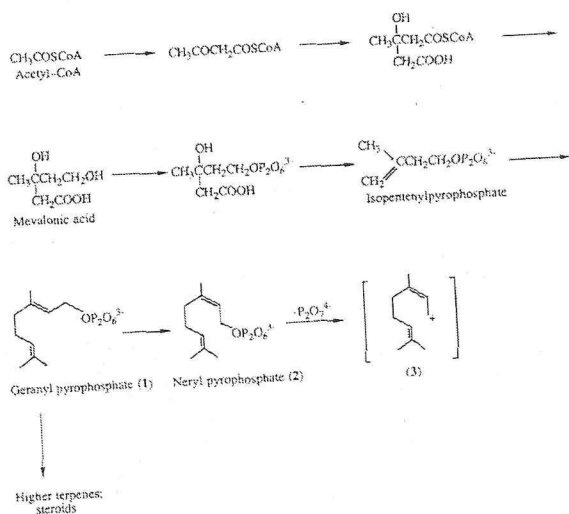


Figure 1. The biosynthetic pathway from acetate, in the form of acetyl coenzyme-A, via geranyl pyrophosphate, to the carbocation intermediate (3).

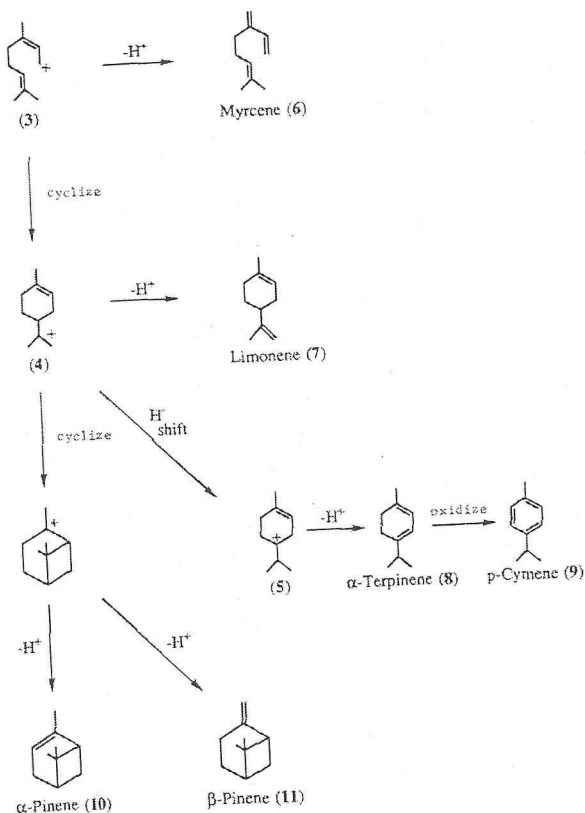
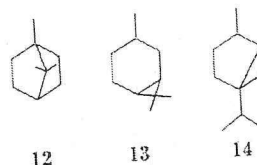


Figure 2. Pathways for the conversion of the carbocation (3) to a range of simple monoterpene hydrocarbons (6-11).

range of acyclic, monocyclic, or bicyclic products. In particular, as shown in Figure 2, simple carbocation reactions of (3), such as C—C bond formation, hydride shifts, and deprotonation can lead via the intermediates (4) and (5) to a whole range of steam volatile hydrocarbons, of which those in Figure 2 (6-11) are of common occurrence: other similar cyclizations of the bornane (12), carane (13), or thujane (14) frameworks, respectively.



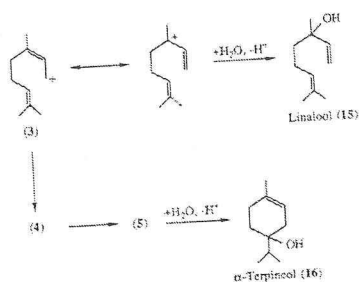
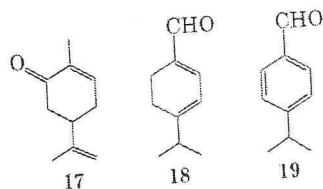


Figure 3. Pathways for the conversion of the carbocation (3) to the alcohols 15 and 16.

Alternative processes (Fig. 3) can produce alcohols, such as linalool (15) or α -terpineol (16), while oxidation of (7), (8), and (9), respectively, can provide carvone (17), 1,3-menthadien-7-al (18), and cuminaldehyde (19).

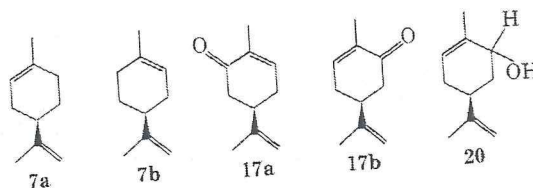


Using this chemistry, we have developed an experiment based upon the steam volatility of simple monoterpenes for use with first-year classes in biological chemistry. This experiment involves not only isolation and purification of the steam-volatile terpene fraction but also characterization in simple cases by infrared spectroscopy (IR) and gas-liquid chromatography (GLC); as well as providing practice in stereochemical assignment, the experiment exposes the students also to the use of sophisticated coupled gas chromatography/mass spectrometry (GC-MS) and provides some insight into simple biosynthetic transformations. We have used this experiment successfully with large classes of freshman students, all of whom have previously taken an introductory course in chemistry (3), which for the majority of our classes has been their first experience of "hands-on" experimental work in chemistry, during which they learn the basic manipulative skills, the use of instrumentation such as IR and GLC, and the elements of spectral interpretation.

Each student undertakes the steam distillation of some readily available materials: fresh orange peel, ground caraway seeds, or ground cumin seeds. After isolation of the steam-volatile organic fractions, these are analyzed by GLC, and the products from orange peel and caraway seeds are identified by GLC comparison with authentic samples of simple terpenes: quantitative analysis of any mixed products is also undertaken using GLC, and IR spectra are recorded. For the product from cumin seeds, students run their own GLC traces and are then provided with the output from a GC-MS analysis, in which automatic library search and comparison methods have been used for identification of the major components: this brings the students, at an early stage, into contact with contemporary analytical practice.

Fresh orange peel provides almost pure $R(+)$ limonene (7a), while caraway seeds provide a mixture of 7a and $S(+)$ carvone (17a) with a typical molar ratio of ~1:3. When the students have identified these products, they are provided with two pure limonene samples, one from oranges and the other $S(-)$ limonene (7b) from pine needles, and are asked to compare the odors of these spectroscopically and chromatographically identical substances: they are similarly

provided with two pure carvone samples, $S(+)$ carvone (17a) from caraway and $R(-)$ carvone from spearmint, and again asked to compare the odors; they also draw both enantiomers for limonene and carvone, assigning them as R or S .



The enzyme system that mediates the cyclization of 3, or of some masked equivalent of 3, or of some masked equivalent of 3, to limonene (7), must therefore be different in oranges and pine, since these plants provide the opposite enantiomers. It is probable that limonene (7) is in fact a biogenic precursor of carvone (17) as judged by feeding studies using ^{14}C -labelled substrates (1), with carveol (20) an intermediate. Formation thus of S -carvone from R -limonene would require no stereochemical change at the chiral center, and it is just such a mixture that is isolated from caraway seeds.

Although $S(-)$ limonene (7b) can be obtained from pine needles and $R(-)$ carvone (17b) from spearmint leaves, we choose to perform the extractions using orange peel and caraway seeds simply because these materials are easier to obtain on a year-round basis.

In contrast to these two simple steam distillates, that from ground cumin seeds presents a rather more complex mixture of products. Five major and two minor components can be identified: the major products, in order of increasing GLC retention times, are β -pinene (11), p -cymene (9), α -terpinene (8), cuminaldehyde (19), and 1,3-menthadien-7-al (18), together with minor quantities of α -pinene (10) and α -terpineol (16). Traces of C_{15} sesquiterpenes are also detectable. These identifications, made using GC-MS with computerized library search, accord with those made in several previous studies (4-6); they differ from at least one study (7) that reported limonene (7) and α -phellandrene (21) as components of cumin oil.



When the orange peel extract described earlier was subjected to the more searching examination provided by the GC-MS technique, using very high amplification, traces only of myrcene (6) and linalool (15) were also detected: under similar conditions, no substances other than (7) and (17) were detected in caraway extract.

Thus, between them these three extracts contain all the neutral hydrocarbons (6)-(11) in Figure 2 and all the simple oxygenated derivatives (15-19). While the predominant contributors to the characteristic aromas of orange peel and caraway extracts are $R(+)$ limonene (7a) and $S(+)$ carvone (17a), respectively, the characteristic odor of steam-extracted cumin oil is provided (5) predominantly by the two aldehydes 18 and 19.

Experimental

Extraction of Orange Peel

Weigh ~100 g of fresh orange peel, and chop it into small pieces using a sharp knife. Place the chopped peel in a 500-mL round-bottomed flask, add a few antipumping granules and 250 mL water.

Fit an antispash stillhead, water condenser, receiver adaptor, and a 250-mL flask as receiver.

Heat the mixture with a Bunsen burner, and distill over ~150 mL of distillate. Extract the aqueous distillate with CH_2Cl_2 (5 × 20 mL); combine the CH_2Cl_2 fractions, and dry over anhydrous MgSO_4 . Filter, and then remove the solvent. Weigh the product, note its odor, and record its IR spectrum.

Extraction of Caraway or Cumin Seeds

Weigh ~50 g of dry seed, and grind in an electric blender. Carry out the steam distillation, CH_2Cl_2 extraction, and isolation precisely as for the orange peel extraction. Again weigh each product, noting the odor, and record the IR spectra.

GLC Analysis

Prepare dilute solutions (1:20 v/v) of each product in dry CH_2Cl_2 . Record GLC traces for each, and for similar solutions of authentic limonene and carvone, using a 3% APL column at 150 °C; record retention times, identifying as many components as possible, and the molar proportions of mixed products.

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