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Article

Kováts Retention Indexes of Monounsaturated C_{12}, C_{14}, and C_{16} Alcohols, Acetates and Aldehydes Commonly Found in Lepidopteran Pheromone Blends.

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Muitos feromônios de lepidópteras são constituídos por misturas de álcoois, aldeídos e acetatos, monoinsaturados. O fato de tais compostos normalmente estarem presentes em escala de nanogramas em extratos obtidos das glândulas de lepidópteras, praticamente descarta a possibilidade de se empregar métodos espectroscópicos clássicos na identificação estrutural dos mesmos. A utilização de índices de retenção obtidos via cromatografia gasosa, em combinação com os dados acumulados através da análise do extrato via CG-EM e eletroantenografia, fornecem importantes informações sobre a estrutura de substâncias presentes nos extratos em pequenas quantidades. A comparação dos índices de Kováts (KIs) de substâncias desconhecidas com padrões pode levar à identificação das mesmas ou, ao menos, limitar o número de possíveis estruturas. A alta reprodutibilidade dos KIs possibilita que comparações sejam feitas através de valores determinados em qualquer laboratório, sem a necessidade de se ter os padrões. Nesse trabalho foram calculados os KIs de quase todos os possíveis isômeros monoinsaturados de álcoois, aldeídos e acetatos de 12, 14 e 16 átomos de carbono, analisados em colunas de fase estacionária apolar (DB-5) e polar (DB-WAX).

Many lepidopteran pheromones consist of blends of monounsaturated alcohols, aldehydes and acetates. These compounds frequently are obtained in only nanogram quantities from pheromone gland extracts, prohibiting use of most standard spectroscopic methods. However, use of GC retention indexes, particularly in combination with mass spectrometry and electroantennograms, can provide substantial information about trace amounts of unknowns. Comparison of Kováts indexes (KIs) of an unknown with those of standards can provide an unambiguous identification, or at least, limit the number of possible structures to a few compounds. Furthermore, because KIs are highly reproducible, a full set of standards is not necessary; comparisons can be made using tables of KIs determined in another laboratory. We provide here tables of the KIs of almost all possible isomers of monounsaturated alcohols, aldehydes, and acetates with 12, 14, or 16 carbon chain lengths, measured on nonpolar (DB-5) and polar (DB-WAX) stationary phases.

Keywords: Kováts retention index, pheromone, Lepidoptera

Introduction

To date, pheromone blends have been identified for more than 1,000 lepidopteran species[1]. A substantial fraction of the components that comprise these blends are monounsaturated straight-chain alcohols, aldehydes and acetates with chain lengths of 12, 14, or 16 carbons. These compounds are usually found in amounts that vary from picograms to micrograms per individual, depending on species. Consequently, they are rarely isolated in quantities sufficient to carry out the full range of spectroscopic tests (^{1}H and ^{13}C NMR, infrared, and mass spectrometry) normally used to characterise organic compounds. Instead, identifications usually have relied on mass spectrometry (MS), and particularly MS coupled with gas chromatography, which provides both the separation of most compounds in a crude extract of the pheromone glands, and yields a full scan mass spectrum on any compound present in amounts of a few nanograms or more. Microchemical tests also may be used to determine the presence or absence of specific functional groups[2]. However, the amount of information that can be obtained from these methods is somewhat limited, and conducting microchemical tests on nanogram to picogram quantities

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of compounds without losing them altogether can be technically challenging. The problem is exacerbated by the fact that whereas mass spectrometry can frequently be used to determine the gross structure of a monounsaturated alcohol, aldehyde, or acetate pheromone component, in general the mass spectrum provides insufficient information to determine either the position or the geometry of the double bond reliably; for example, the mass spectra of most monounsaturated acetates of a given chain length are quite similar. Furthermore, because different types of mass spectrometers (*e.g.*, quadrupole, magnetic sector, or ion trap) produce slightly different spectra, matchups with literature spectra cannot be relied upon for determinations of double bond positions or geometries.

Because of these problems, GC retention time data has been used extensively in the identification of lepidopteran pheromones, with matching retention times between an unknown compound and a standard of known structure providing strong support for a tentative identification. However, a retention time match is not conclusive, because isomers may have very similar retention times. Nevertheless, retention time matches, particularly on two or more GC columns of differing polarity, can at least narrow the possibilities to a few structures, providing that standards of all possible structures are available for comparison. This is frequently not the case, particularly for laboratories that have not accumulated a library of standards.

The necessity of having a full set of standards can be circumvented by careful use of retention index data, because retention indexes, within a small margin of error, are a function only of the GC column and the operating conditions. As long as these are faithfully reproduced, the retention index of an unknown compound can be replicated in any laboratory. Thus, calculation of the retention index of an unknown compound and comparison with tabulated values of standards run under the same conditions provides a piece of data of enormous value in the identification of an unknown. At the very least, comparison of retention indexes usually allows all but a few isomers to be eliminated from consideration. The most widely used system of retention indexes is that introduced by Kováts[3], in which retention indexes are calculated relative to straight-chain alkane standards. Other sets of standards have also been used to a lesser extent, such as saturated fatty acid methyl esters, or acetate esters of alkanols[4].

Although tables of retention index data have probably been compiled by laboratories that work frequently with lepidopteran pheromones, to our knowledge these compilations have not been published. To remedy this deficiency, and to provide a resource for laboratories that cannot obtain extensive libraries of standards, we provide here the Kováts retention indexes (KI's) of most of the isomers of monounsaturated C_{12}, C_{14}, and C_{16} alcohols, aldehydes and acetates, determined on both nonpolar and polar GC stationary phases.

Experimental

Preparation of standards for analysis

The monoene alcohols, aldehydes and acetates were taken from our library of standards, assembled over many years from a number of sources, including The Pherobank (Research Institute for Plant Protection, Wageningen, The Netherlands) and the collection of the Plant Biotechnology Institute (National Research Council of Canada, Saskatoon, Sask., Canada). Stock solutions of alcohols and acetates in dichloromethane (~1mg cm^{-3}) were checked for purity by thin-layer chromatography on silica gel (E. Merck, Type 5554 plates; Fisher Scientific, Pittsburgh, PA). When necessary, compounds were purified by chromatography on silica gel as follows. Solutions in CH_2Cl_2 (0.2 cm^3, 1 mg cm^{-3}) were added to a Pasteur pipet packed with silica gel (3.5 cm). Compounds were eluted with mixtures of hexane/ethyl acetate (alcohols, 4:1; acetates, 19:1), collecting 1 cm^3 fractions. Fractions contained the desired compound were combined and concentrated under reduced pressure to ~0.05 cm^3.

Aldehydes were prepared by oxidation of the corresponding alcohols. Thus, pyridinium chlorochromate (PCC, 500 mg) and powdered 4Å molecular sieves (500 mg) were ground to fine powder and the mixture (10 mg) was added to a vial with CH_2Cl_2 (0.15 cm^3). The alcohol solution in CH_2Cl_2 (0.05 cm^3, *ca.* 1mg cm^{-3}) was added and the flask was manually agitated every 5 min for 30 min. Heptane (0.8 cm^3) was then added, the vial was vortexing for 10 s, and the precipitate was left to settle for 10 min. The mixture was then filtered through a Pasteur pipet packed with powdered $MgSO_4$ (2.5 cm).

PCC oxidation of homoallylic alcohols to the corresponding aldehydes was not successful due to extensive isomerization. Instead Collin's oxidation was used; the oxidation of (*E*)-3-tetradecenol is representative. Chromium trioxide (28 mg, 0.28 mmol) was added to a stirred solution of pyridine (45 mg, 0.56 mmol) in CH_2Cl_2 (0.25 cm^3). After 15 min a solution of (*E*)-3-tetradecenol in CH_2Cl_2 (0.1 cm^3, 1 mg cm^{-3}) containing n-propanol (2.8 mg, 0.047 mmol) was added. After stirring 15 min at room temp, heptane (0.7 cm^3) was added, the vial was vortexed and the precipitate was left to settle for 10 min. The liquid phase was filtered through a pipette packed with powdered $MgSO_4$.

To confirm the positions of double bonds in selected compounds, dimethyldisulfide (DMDS) adducts were made for GC-MS analysis[2]. Thus, a solution of DMDS in hexane (1 cm^3, 10% v/v) was mixed with a solution of iodine in ether (0.4 cm^3, 50 mg cm^{-3}). An aliquot of the resulting solution (0.1 cm^3) was added to a solution of a monoene in CH_2Cl_2 (0.05 cm^3, 1mg cm^{-3}) and heated for 20 h at 55ºC in a tightly closed screw-cap vial. Sodium thiosulphate solution (5% w/v) was added dropwise to the cooled solution with vortexing until the mixture was colorless. Pentane (0.5 cm^3) was then added and the mixture was vortexed for 10 s. The organic phase was transferred to a clean vial and the solution was concentrated under a stream of nitrogen for GC-MS analysis.

Analysis conditions

For efficiency, mixtures of the alcohol, aldehyde, and acetate of the same chain lengths, double bond position, and geometry were analyzed as a single solution, in final concentrations of ~ 50 to 100 μg cm^{-3}, with C_{13}-C_{28} hydrocarbons added (5 to 10 μg cm^{-3}) for retention index calculations. Compounds were analyzed by GC in splitless mode on a Hewlett-Packard 5890A or 5890 Series II GC (HP, Palo Alto CA), equipped with either a DB-5 column (30 m x 0.25 mm ID, 0.25 μm film, J&W Scientific, Folsom CA; temp. program 100ºC/1min, 5ºC/min to 275ºC for 3 min, 128 kPa head pressure, 29.4 cm/sec linear velocity) or a DB-WAX column (30 m x 0.32 mm ID, 0.25 μm film, J&W Scientific; temp. program 100ºC/1min, 5ºC/min to 240ºC for 0 min, 138 kPa head pressure, 43 cm/s linear velocity). Selected compounds were also analyzed by GC-MS using an HP 5890 GC interfaced to an HP 5970B mass selective detector in electron impact ionization mode (70 eV). The GC-MS was equipped with a DB-5MS column (20 m x 0.2 mm ID), using a temperature program of 100ºC/ 1 min, 10ºC/min to 250ºC for 5 min. Helium was used as carrier gas for all GC and GC-MS analyses. The following solutions containing alcohols, aldehydes and acetates were analyzed by GC-MS to verify the gross structures of one or more of the constituents: 6*E*-12, 5*Z*-12, 4*Z*-12, 4*E*-12, 3*Z*-12, 3*E*-12, 3*E*-14, 3*Z*-14, 3*Z*-16. Another subset of single-component samples, including 4*Z*-12:OH, 3*E*-12:OH, 3*E*-14:OH, 7*E*-16:OH, 11*E*-16:OH, 4*E*-16:OAc, were derivatized with DMDS as described above and analyzed by GC-MS to confirm the double bond positions.

Kováts retention indexes of each compound on each column were calculated using the formula[5]:

$$KI = 100y + 100(z-y)\ X\ \frac{t_{r(x)} - t_{r(y)}}{t_{r(z)} - t_{r(y)}}$$

where y and z are the carbon numbers of the hydrocarbon standards on either side of a given compound, $t_{r(x)}$ is the retention time of the compound, and $t_{r(y)}$ and $t_{r(z)}$ are the retention times of the hydrocarbon standards. The specific hydrocarbon standards used in the calculation of the retention indexes of each group of compounds are listed in the table and figure captions.

Results and Discussion

DB-5 (Tables 2-4, Figures 1-3) and DB-WAX (Tables 5-7, Figures 4-6) bonded stationary phases were chosen for calculation of KI values because they represent typical examples of the common nonpolar 5% phenyl-95% dimethylpolysiloxane and polar Carbowax (polyethyl-eneglycol) stationary phases that are available from all major suppliers of capillary GC columns (see Table 1 for list of equivalent columns from other manufacturers). KI values calculated on any of these equivalent columns will be close to the values reported here, as will the overall shapes of plots of KI versus double bond position. Furthermore, retention indexes were calculated using temperature programmed rather than isothermal analyses for two reasons. First, crude extracts of pheromone glands contain numerous compounds of different volatility, so temperature programming is required in order to elute all compounds of interest within a timely fashion, and without significant peak broadening for later-eluting compounds. Second, with temperature programming, the equation for calculation of Kováts indexes simplifies considerably[5]: with isothermal runs, the equation requires use of the logarithms of the adjusted retention times, whereas with temperature programming, the relationship between retention time and retention index becomes approximately linear, so that the logarithmic terms in the equation can be replaced with the simple retention times. In theory, as long as the same stationary phase and operating conditions are used, retention indexes should be highly reproducible between laboratories. In practice, slight variability between laboratories may occur due to aging of stationary phases with use and other factors. Nevertheless, it has been estimated that if operating conditions are reproduced carefully, interlaboratory variations of no more than 2-5 retention index units are easily attainable[5]. This variability can probably be decreased further if one or more standards of the alkenes in questions are available as markers. That is, by measuring the retention indexes of the standards and determining how much they differ from the tabulated values presented here, the expected values of the remaining compounds can be adjusted accordingly.

Table 1. Cross reference table of capillary GC stationary phases similar to DB-5 and DB-WAX, available from different manufacturers.

Manufacturer	DB-5 equivalent	DB-WAX equivalent
Restek	RTX-5	RTX-WAX, Stabilwax
Supelco	SPB-5	Supelcowax 10
Hewlett-Packard /Agilent	HP-5, Ultra-2	HP-20M
Alltech	ATF-5	AT-WAX
SGE	BP-5, BPX-5	BP-20
Chrompack	CP sil 8 CB	CP-WAX 52 CB

Table 3. Kováts indexes of C_{14} monoene aldehydes, alcohols, and acetates on the DB-5 column*.

	Aldehyde		Alcohol		Acetate	
Bond position	*E*	*Z*	*E*	*Z*	*E*	*Z*
saturated	1615		1677		1811	
2	1674**	1674**				
3	1601	1599	1663	1662	1796	1791
4	1602	1599	1664	1660	1796	1787
5	1599	1594	1665	1662	1795	1790
6	1599	1597	1662	1660	1796	1789
7	1600	1597	1663	1660	1797	1792
8	1601	1599	1665	1663	1798	1796
9	1604	1603	1668	1667	1801	1801
10	1606	1609	1669	1673	1803	1807
11	1610	1615	1673	1678	1810	1812
12	1619	1632	1682	1695	1817	1831
13	1608		1670		1805	

* Hydrocarbon standards used: for aldehydes, C_{15} and C_{17}, for alcohols, C_{16} and C_{17}, for acetates, C_{17} and C_{19}; ** Because of facile isomerization of α,β-unsaturated aldehydes, the stereochemistry of the double bond is uncertain.

Table 5. Kováts indexes of C_{12} monoene aldehydes, alcohols, and acetates on the DB-WAX column*.

	Aldehyde		Alcohol		Acetate	
Bond position	*E*	*Z*	*E*	*Z*	*E*	*Z*
saturated	1700		1970		1880	
2	1844**	1846**				
3	1736	1736	1965	1989	1894	1896
4	1732	1733	1995	1996	1899	1891
5	1725	1724	2003	2007	1904	1901
6	1731	1734	2001	2007	1905	1905
7	1732	1737	2004	2009	1908	1911
8	1733	1743	2005	2016	1910	1920
9	1742	1752	2013	2024	1920	1929
10	1763	1781	2035	2054	1942	1961
11	1753		2023		1930	

* Hydrocarbon standards used: for aldehydes, C_{17} and C_{19}, for alcohols, C_{19} and C_{21}, for acetates, C_{18} and C_{20}; * Because of facile isomerization of α,β-unsaturated aldehydes, the stereochemistry of the double bond is uncertain.

Table 2. Kováts indexes of C_{12} monoene aldehydes, alcohols, and acetates on the DB-5 column*.

	Aldehyde		Alcohol		Acetate	
Bond position	*E*	*Z*	*E*	*Z*	*E*	*Z*
saturated	1407		1473		1609	
2	1466**	1467**				
3	1399	1399	1451	1457	1593	1591
4	1396	1393	1460	1457	1596	1589
5	1394	1389	1464	1461	1597	1592
6	1396	1394	1462	1461	1598	1593
7	1397	1396	1465	1463	1600	1598
8	1399	1401	1466	1468	1602	1605
9	1403	1407	1470	1473	1607	1611
10	1412	1424	1478	1490	1615	1630
11	1401		1466		1604	

* Hydrocarbon standards used: for aldehydes, C_{13} and C_{15}, for alcohols, C_{14} and C_{15}, for acetates, C_{15} and C_{17}; ** Because of facile isomerization of α,β-unsaturated aldehydes, the stereochemistry of the double bond is uncertain.

Table 4. Kováts indexes of C_{16} monoene aldehydes, alcohols, and acetates on the DB-5 column*.

	Aldehyde		Alcohol		Acetate	
Bond position	*E*	*Z*	*E*	*Z*	*E*	*Z*
saturated	1819		1882		2013	
2	1880**	1879**				
3	1808	1807	1865	1868	1994	1994
4	1806	1804	1868	1865	2000	1987
5	1803	1799	1869	1867	1995	1989
6	1803	1799	1866	1863	1997	1987
7	1804	1798	1871	1862	1995	1988
8	1803	1798	1866	1862	1995	1990
9	1804	1800	1868	1863	1998	1993
10	1806	1804	1869	1867	2000	1998
11	1810	1809	1877	1874	2004	2004
12	1813	1816	1877	1879	2008	2011
13	1815	1820	1878	1884	2010	2016
14	1824	1838	1887	1902	2018	2033

* Hydrocarbon standards used: for aldehydes, C_{17} and C_{19}, for alcohols, C_{18} and C_{20}, for acetates, C_{19} and C_{21}; ** Because of facile isomerization of α,β-unsaturated aldehydes, the stereochemistry of the double bond is uncertain.

Table 6. Kováts indexes of C_{14} monoene aldehydes, alcohols, and acetates on the DB-WAX column*.

	Aldehyde		Alcohol		Acetate	
Bond position	*E*	*Z*	*E*	*Z*	*E*	*Z*
saturated	1910		2177		2087	
2	2059**	2060**				
3	1940	1945	2201	2196	2104	2101
4	1940	1940	2201	2201	2104	2093
5	1932	1930	2207	2209	2108	2103
6	1936	1937	2203	2207	2107	2103
7	1935	1937	2204	2206	2108	2108
8	1938	1940	2207	2210	2111	2113
9	1942	1946	2211	2215	2116	2120
10	1943	1954	2212	2223	2118	2128
11	1953	1963	2221	2232	2129	2137
12	1976	1994	2243	2263	2150	2169
13	1964		2231		2137	

* Hydrocarbon standards used: for aldehydes, C_{19} and C_{21}, for alcohols, C_{21} and C_{23}, for acetates, C_{20} and C_{22}.
** Because of facile isomerization of α,β-unsaturated aldehydes, the stereochemistry of the double bond is uncertain.

Table 7. Kováts indexes of C_{16} monoene aldehydes, alcohols, and acetates on the DB-WAX column*.

	Aldehyde		Alcohol		Acetate	
Bond position	*E*	*Z*	*E*	*Z*	*E*	*Z*
saturated	2120		2385		2294	
2	2279**	2278**				
3	2158***	2158***	2384	2407	2307	2310
4	2151	2151	2409	2410	2314	2300
5	2143	2140	2415	2417	2314	2308
6	2146	2145	2410	2412	2313	2306
7	2144	2144	2413	2410	2312	2308
8	2144	2144	2411	2411	2314	2312
9	2146	2147	2413	2413	2317	2315
10	2150	2152	2416	2419	2321	2322
11	2156	2159	2423	2427	2325	2330
12	2157	2168	2422	2433	2328	2339
13	2166	2177	2430	2441	2337	2347
14	2189	2210	2451	2472	2359	2379

* Hydrocarbon standards used: for aldehydes, C_{21} and C_{24}, for alcohols, C_{22} and C_{25}, for acetates, C_{22} and C_{24}.
** Because of facile isomerization of α,β-unsaturated aldehydes, the stereochemistry of the double bond is uncertain.
*** Value not reliable due to on-column isomerization/tautomerization.

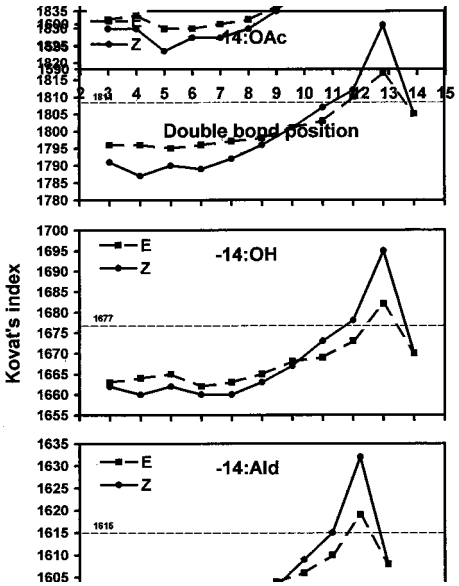

Figure 2. Plots of Kováts retention indexes determined on DB-5 stationary phase versus double bond position for C_{14} monounsaturated aldehydes, alcohols, and acetates. Hydrocarbon standards used: for aldehydes, C_{15} and C_{17}, for alcohols, C_{16} and C_{17}, for acetates, C_{17} and C_{19}. The dashed line indicates the retention index of the saturated analogs.

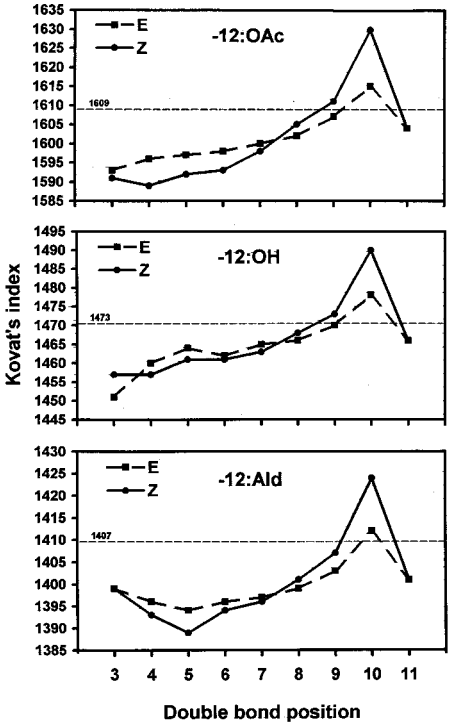

Figure 1. Plots of Kováts retention indexes determined on DB-5 stationary phase versus double bond position for C_{12} monounsaturated aldehydes, alcohols, and acetates. Hydrocarbon standards used: for aldehydes, C_{13} and C_{15}, for alcohols, C_{14} and C_{15}, for acetates, C_{15} and C_{17}. The dashed line indicates the retention index of the saturated analogs.

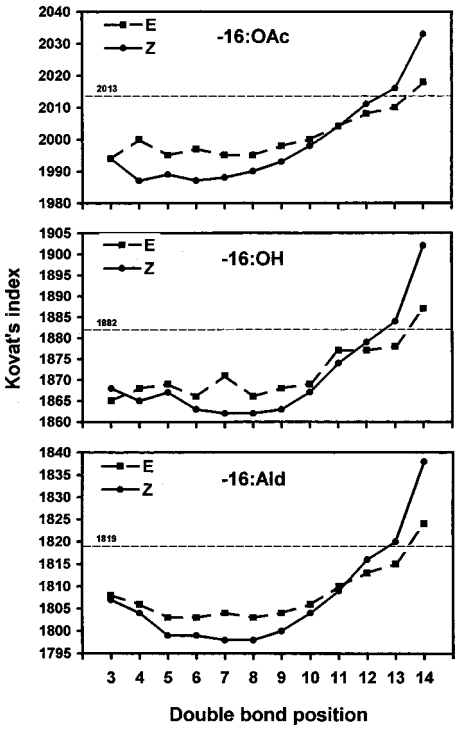

Figure 3. Plots of Kováts retention indexes determined on DB-5 stationary phase versus double bond position for C_{16} monounsaturated aldehydes, alcohols, and acetates. Hydrocarbon standards used: for aldehydes, C_{17} and C_{19}, for alcohols, C_{18} and C_{20}, for acetates, C_{19} and C_{21}. The dashed line indicates the retention index of the saturated analogs.

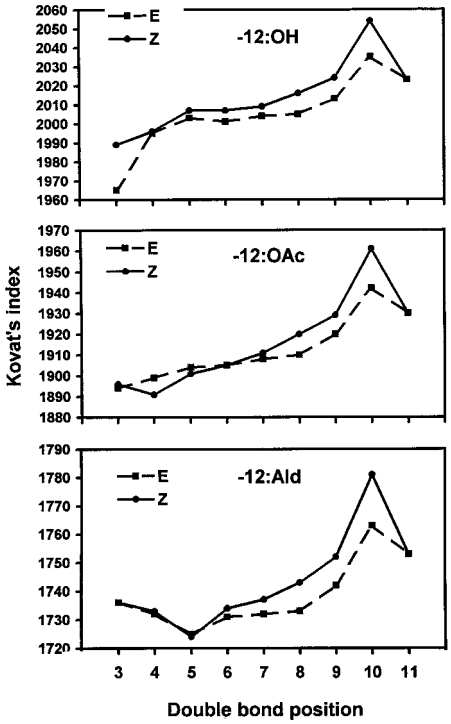

Figure 4. Plots of Kováts retention indexes determined on DB-WAX stationary phase versus double bond position for C_{12} monounsaturated aldehydes, alcohols, and acetates. Hydrocarbon standards used: for aldehydes, C_{17} and C_{19}, for alcohols, C_{19} and C_{21}, for acetates, C_{18} and C_{20}.

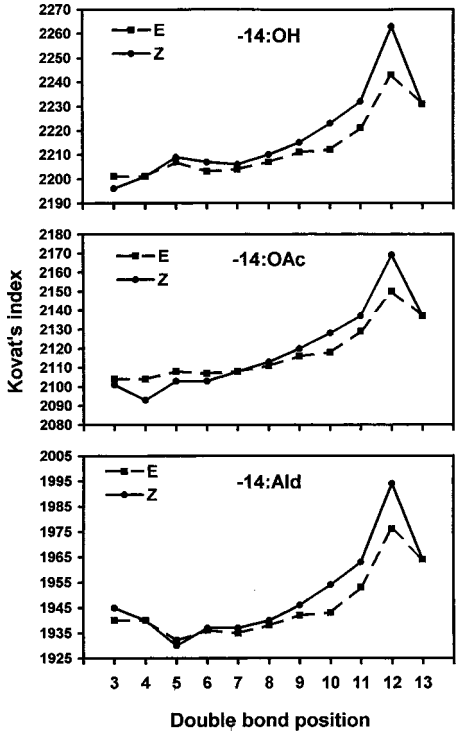

Figure 5. Plots of Kováts retention indexes determined on DB-WAX stationary phase versus double bond position for C_{14} monounsaturated aldehydes, alcohols, and acetates. Hydrocarbon standards used: for aldehydes, C_{19} and C_{21}, for alcohols, C_{21} and C_{23}, for acetates, C_{20} and C_{22}.

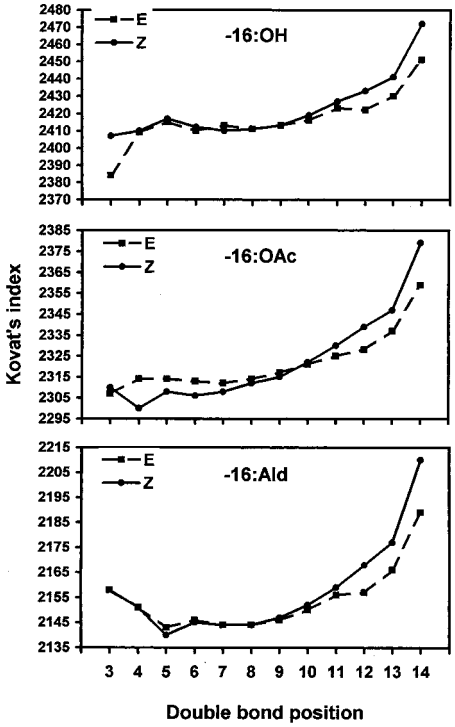

Figure 6. Plots of Kováts retention indexes determined on DB-WAX stationary phase versus double bond position for C_{12} monounsaturated aldehydes, alcohols, and acetates. Hydrocarbon standards used: for aldehydes, C_{21} and C_{24}, for alcohols, C_{22} and C_{25}, for acetates, C_{22} and C_{24}.

Comparisons of the retention behavior of a compound on polar and nonpolar stationary phases can provide significant amounts of information about the structure. First, an alkene with one or more isolated double bonds usually has a retention time shorter than that of the saturated analog on a nonpolar column, whereas an alkene with the double bond(s) anywhere in the molecule always increases the retention time on a polar column relative to the saturated compound. Second, conjugation of an alkene double bond with a carbonyl or with another carbon-carbon double bond always increases the retention time on both nonpolar and polar columns relative to the saturated analog. Third, the elution order of aldehydes, alcohols, and acetates of the same chain length changes between DB-5 and DB-WAX columns. On nonpolar DB-5 (Tables 2-4, Figures 1-3), retention indexes increase in the order aldehyde, alcohol, acetate, whereas on DB-WAX (Tables 5-7, Figures 4-6), the elution order is aldehyde, acetate, alcohol. Fourth, the differences in retention indexes between two compounds can provide useful information. For example, on DB-5 (Tables 2-4, Figures 1-3), an alcohol and an acetate of the same chain length have KI values approximately 50-70 units and 190-205 units larger respectively than that of the corresponding aldehyde. On DB-WAX (Tables 5-7, Figures 4-6), these differences in KI

values range from approximately 260-280 (alcohol versus aldehyde) and 160-180 (acetate versus aldehyde).

Plots of KI values versus double bond position were similar for all three functional groups and all three chain lengths, on both the nonpolar (DB-5, Figures 1-3) and polar (DB-WAX, Figures 3-6) stationary phases respectively. KI values generally increased as the double bond was moved from position 3 to position n-3 (where n = the chain length), increased sharply at position n-2, and then dropped sharply for the terminal double bond. On DB-5 (Figures 1-3), for compounds with the double bond closer to the functional group than to the distal end of the molecule, *Z*-isomers almost always had smaller KIs than the corresponding *E*-isomers, but this trend reversed as the double bond was moved towards the distal end of the chain. On DB-WAX (Figures 4-6), there was generally little difference between the KIs of compounds with the double bond closer to the functional group than to the distal end of the molecule, with the exception of 3-alkenols, in which the KIs of the *E*-isomers were considerably lower than those of the *Z*-isomers for the C_{12} and C_{16} compounds. For compounds with the double bond in the latter half of the chain, the KIs of the *Z*-isomers was always higher than that of the corresponding *E*-isomers.

Looked at on finer scale, there were some apparent anomalies, such as the noticeable decrease in KI values for compounds such as *Z*5-12:Ald (Figures 1and 4), *Z*4-14:OAc (Figures 2 and 5), and *Z*5-14:Ald (Figures 2 and 5) on both DB-5 and DB-WAX. However, these apparent anomalies were determined to be real by a) running the samples again, b) checking the anomolous samples by MS to ensure that the gross structure was correct, and c) forming the DMDS adducts of the compounds, followed by MS analysis to unambiguously determine the position of the double bond. In all cases, the structures were confirmed. In fact, these anomalies can be very useful by providing a distinct break in a general trend, and thus providing a KI value for which there can be fewer possible structures. For example, the position and geometry of the double bond in *Z*5-12:Ald or *Z*4-14:OAc can be determined from their KI values on DB-5 alone. In analogous fashion, the KI values of *Z*4-14:OAc and *Z*4-16:OAc on DB-WAX unambigously determine the double bonds in these two compounds. Overall, in the best case, the combination of KI values of any given compound on the two columns would allow the reliable assignment of the double bond position and geometry in an unknown compound. Even in the worst case, careful use of the retention indexes limits the number of possibilities to only a few isomers from the many possible, greatly reducing the number of compounds that must be synthesized to confirm the identification. Furthermore, other data, such as careful comparison of the mass spectrum of the unknown with those of some of the possible structures, or testing the antennal responses of male moths to possible structures, can further narrow the possibilities, frequently to only a single compound.

The power of retention indexes for structure identification can be illustrated best with several case studies from the recent literature. For example, extracts of the sex pheromone gland of the African white rice stem borer *Maliarpha separatella* (Lepidoptera: Pyralidae)[4] contained *Z*9-14:OH, *Z*9,*E*12-14:OH, and *E*10,*E*12-14:OH, in a ratio of 2: 3.5: 1. The average amount of the major component per insect was only 0.4 ng, providing only enough material for determination of retention times, and EI and CI mass spectra. By careful use of retention indexes, the identities of all three compounds were unambiguously determined, and subsequently corroborated in field bioassays. Interestingly, *Z*9-14:OH inhibited attraction of male moths, suggesting that it is not a component of the pheromone even though it was present in the pheromone gland.

In studies of the soybean pod borer *Etiella behrii* (Lepidoptera: Pyralidae)[6], four compounds in pheromone gland extracts were found to stimulate male moth antennae in electroantennogram studies. The amounts of the active components (<0.2 ng/female) were insufficient to obtain any spectral data, and consequently identifications were carried out entirely by use of retention indexes, determined on polar (DB-WAX) and nonpolar (HP-1) columns. Based solely on this information, 3 of the 4 compounds were identified unambiguously as 12:OAc, *E*9-12:OAc, and *Z*11-14:OAc, and the identity of the 4th component was restricted to 2 possibilities, either *Z*9- or *E*11-14:OAc. The identity of the 4th component was verified as *E*11-14:OAc by field tests.

In an extreme example, extracts of pheromone glands from females of the leafminer moth *Lyonetia prunifoliella* (Lepidoptera: Lyonetiidae) contained less than 10 picograms per female of 2 of the 3 components of the pheromone, and approximately 50 picograms of the major component[7]. The former 2 components were present in such small amounts that they produced no visible peaks in gas chromatograms. However, analysis of the extracts by coupled gas chromatography-electroantennogram detection, using male moth antennae as detectors, produced signals from the male moth antennae at the retention times of the biologically active compounds. Retention indexes of the two minor components determined on DB-5, DB-23, and DB-210 columns were all indicative of methyl-branched alkanes, several examples of which were known from the Lyonetiidae[1].

Based only on this information, the two compounds were tentatively identified as 5,9-dimethylheptadecane and 5,9-dimethyloctadecane. A mass spectrum obtained for the third, major component, suggested that it was a monounsaturated analog of 5,9-dimethyloctadecane, and this was supported by the fact that this component eluted earlier than 5,9-dimethyloctadecane on the nonpolar DB-5 column, and later on the polar DB-23 and DB-210 columns. This information, coupled with the fact that a methyl-branched octadecene with terminal unsaturation had been reported from another lyonetid species, suggested the structure 10,14-dimethyl-1-octadecene, and this identification was confirmed by mass spectral and retention times matches with an authentic standard, and verified in field trials.

Conclusions

When used methodically, retention indexes can provide crucial data for use in the identification of lepidopteran pheromone components, particularly when the small amounts of materials available do not allow a mass spectrum to be obtained. The power of the method can be extended to picogram and even femtogram quantities by use of live male moth antennae as detectors in coupled gas chromatrography-electroantennography[7,8]. Furthermore, related species frequently produce similar pheromone components, allowing short lists of likely structures to be compiled. Comparison of the retention indexes of these likely structures with the retention indexes of the pheromone components in an extract can rapidly eliminate some structures from further consideration, while retention index matches, particularly on more than one type of stationary phase, provide strong evidence for a correct identification.

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