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INTERNET PROGRAMS FOR DRAWING MOTH PHEROMONE ANALOGS AND SEARCHING LITERATURE DATABASE

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Abstract—An Internet web page is described for organizing and analyzing information about lepidopteran sex pheromone components. Hypertext markup language (HTML) with JavaScript program code is used to draw moth pheromone analogs by combining GIF bitmap images for viewing by web browsers such as Netscape or Microsoft Internet Explorer. Straight-chain hydrocarbons of 5–22 carbons with epoxides or unsaturated positions of *E* or *Z* geometrical configuration with several alternative functional groups can be drawn by simply checking menu bars or checkboxes representing chain length, *E*/*Z* unsaturation points, epoxide position and chirality, and optional functional groups. The functional group can be an aldehyde, alcohol, or ester of formate, acetate, propionate, or butyrate. The program is capable of drawing several million structures and naming them [e.g., (*E*,*E*)-8,10-dodecadien-1-ol and abbreviated as *E*8*E*10–12:OH]. A Java applet program run from the same page searches for the presently drawn structure in an internal database compiled from the Pherolist, and if the component is found, provides a textarea display of the families and species using the component. Links are automatically specified for drawn components if found in the Pherolist web site (maintained by H. Arn). Windowed links can also be made to two other JavaScript programs that allow searches of a web site database with over 5900 research citations on lepidopteran semiochemicals and a calculator of vapor pressures of some moth sex pheromone analogs at a specified temperature. Various evolutionary and biosynthetic aspects are discussed in regard to the diversity of moth sex pheromone components.

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INTRODUCTION

During the last few years, the Internet has become accessible to most scientists throughout the world. The quantity of information available through the Internet, or World Wide Web, is likely to grow, improve in quality, and become ever more important to research productivity and education (Byers, 1999; Zenger and Walker, 2000). Web pages are composed of hypertext markup language (HTML) code and are interpreted by web browsers such as Netscape Communicator and Microsoft Internet Explorer (IE), accounting for 37% and 61%, respectively, of 9394 visitors to the menu page, www.vsv.slu.se/cec/h.htm (during 2000, eXTReMe Tracking). JavaScript is a language, originating from Netscape Comm. Corp., that can be loaded from a web page and run by browsers to achieve a moderate level of interactivity and data processing (Goodman, 1996). Java applets (Sun Microsystems Inc.) are separately compiled programs that are called into action by the HTML or JavaScript code and run by the browser or associated software to yield a true program environment, although with security constraints so that only the screen image can be manipulated (Lemay and Perkins, 1997; Vanhelsuwé et al., 1997; Cadenhead, 1999). The use of java applet programs makes it possible to achieve full interactivity and advanced graphical applications. However, by clever use of bitmapped images, JavaScript can also produce interesting visual effects.

From 1975 to 1991 in the *Journal of Chemical Ecology*, there were 619 articles published on Lepidoptera, which accounted for 29% of the journal's reports (Byers, 1992). During the subsequent nine years, 1992–2000, this figure dropped slightly to 25% of 1840 articles. For all insect pheromone papers (1993–2000), BIOSIS Previews cites 3775 articles with 45.3% on Lepidoptera (nearly all on moths). Due to this large interest in lepidopteran pheromones, it was desirable to make a web page that draws and names most moth pheromone components and analogs by simply selecting checkboxes or buttons controlled by JavaScript code. I also wanted the web page to run a Java applet that would immediately tell the user if the drawn compound was a known pheromone component of one or more species in the Pherolist (Arn et al., 1992; Arn, 2000), the well-known web site covering moth pheromone components (www-pherolist.slu.se/index.html). If a known component is drawn, then the page should allow the user to save time by linking automatically to the specific component in the Pherolist. Another goal was to link the page to a searchable web database on moth literature citations (Byers, 2000) and a calculator of moth analog vapor pressures. The final objective was to discuss the diversity of double bond positions of moth sex pheromone components (Pherolist) and analogs

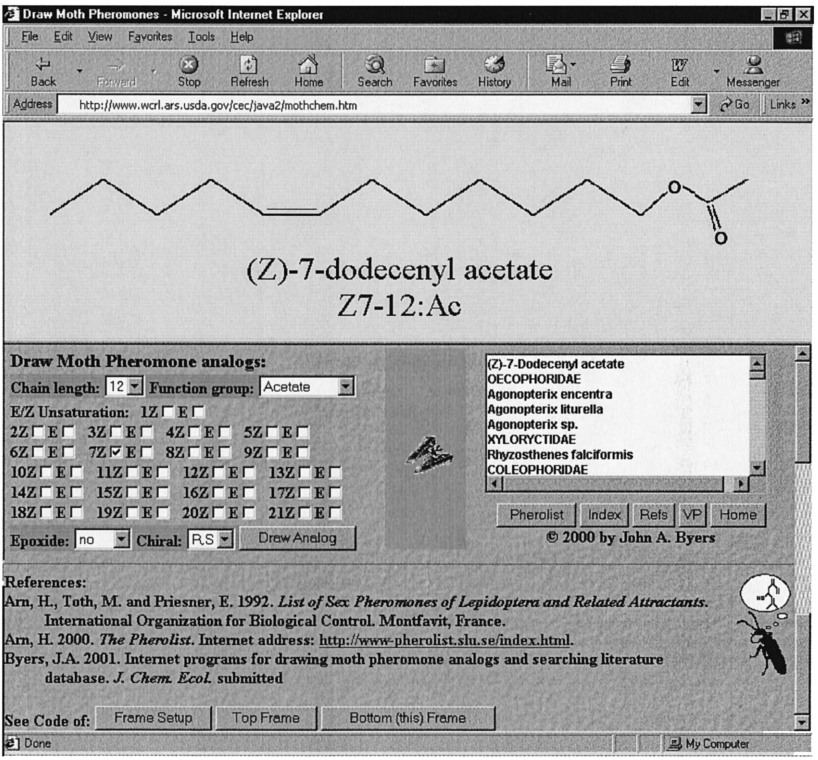

FIG. 1. Internet browser view of frames of web page that draws moth pheromone analogs in top frame based on user input in the bottom frame. Functions of various buttons and textboxes are explained in the text.

(program drawn) in regard to evolutionary and biosynthetic aspects. The web page is at the Internet address: www.wcrl.ars.usda.gov/cec/java2/mothchem.htm (Figure 1).

METHODS AND MATERIALS

The JavaScript code for drawing moth pheromone analogs is found in web frames that can be viewed by Netscape and IE by right-clicking on the frame and selecting "view source." Alternatively, the code for each frame can be viewed from pop-up windows (three buttons at bottom of lower frame, Figure 1).

To draw a molecule, the user selects a chain length from 5 to 22 from a drop-down menu with only one option selection allowed (Figure 1, lower frame). The

same input method is used for selecting the epoxide position and chirality as well as the functional group: either none, an aldehyde, alcohol, or esters of formate, acetate, propionate, and butyrate. A pair of checkboxes for each possible carbon position in the chain can be checked to designate geometrical configuration (*E* or *Z*) of double bonds. Only one box of a pair can be checked. After input is selected, the "draw analog" button is clicked. Several possible error messages may occur if an *E*/*Z* or epoxide position checkbox is selected outside the chain length or is next to position 1 of a functional group. Furthermore, adjacent positions cannot both be unsaturated nor can they be next to or within an epoxide, as these structures are chemically unlikely. Chains with unsaturation at the end opposite the functional group cannot be designated *E* or *Z* (but are delta).

The program then takes the input data and converts them to variables whose values are used to control the drawing of a molecule in the upper frame (Figure 1). The drawing is composed of an appropriate permutation of 22 bitmapped GIF images with transparent backgrounds. These are scaled according to the viewer's screen resolution and displayed from left to right such that the molecule is drawn from the highest numbered carbon toward the functional group. Six of the GIF images represent carbon–carbon bonds, 6 are for epoxides, and 10 are for different configurations of the 6 possible functional groups. The variables are also used to construct the IUPAC name, e.g., (*E*,*E*)-8,10-dodecadien-1-ol, and a short name, *E*8*E*10–12:OH. A further abbreviated name, e.g., e8e1012oh, is made and searched for in an internal database array of about 420 names. If found, as it would be in this case (codlemone), the abbreviation serves as the prefix of the HTML file name in the Pherolist in order to allow automatic linking to this file. The 420-name internal database was made by a BASIC program that extracted pheromone components (and female-produced analogs that could be drawn) from the 544 HTML files (1.5 MB) concerning components that are in the Pherolist as of June 2000.

Another feature found at the right of the lower frame (Figure 1) is a Java applet that is represented by a scrollable text area. The applet consists of a database of component names and the corresponding insect families and species that have or are attracted to the components as extracted from the Pherolist by the BASIC program. Initially the text area informs the user of the purpose of the text area and gives credits to the Pherolist. The data of the applet consists of an array of nonredundant names of families and species in no particular order, but simply as they were encountered in the 544 HTML Pherolist files on chemicals. Each of these files has the component name, as found between the beginning and ending HTML tags of the title, e.g., <TITLE>(E,E)-8,10-dodecadien-1-ol</TITLE>. Similarly, there are unique text strings that indicate the families, genera, and species reported to be attracted to or produce the component. However, the resulting extracted file was larger than 64 KB, the maximum size for a method in Java (version 1.2.2), so the information was indexed using numbers for the array of nonredundant

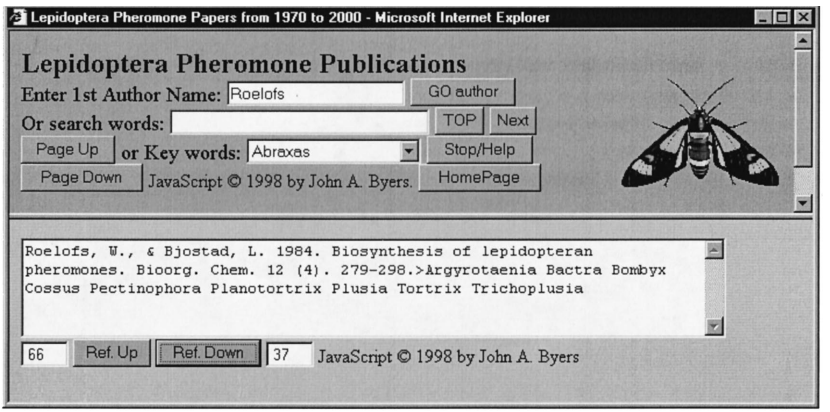

FIG. 2. Internet browser view of frames of a JavaScript database show a search for a specific citation from among currently 5912 references on semiochemicals of Lepidoptera.

species/families mentioned above. This allowed 420 components to be compared to 1716 species and families in the Java applet. Thus, when the user draws a component, e.g., (*E*,*E*)-8,10-dodecadien-1-ol, the Java applet searches its database and shows the names of all families and species attracted by this component (e.g., three subfamilies of Tortricidae and 13 species).

Another feature is a button ("Refs") that, when clicked, allows the user to open a pop-up window with two frames of a web database of citations on over 5900 papers of lepidopteran semiochemicals (Figure 2). A BASIC program similar to that reported earlier (Byers, 1992) reformatted the database, which served as input to another BASIC program that fused templet files of HTML and JavaScript code with the reformatted data to produce 92 web pages with self-searching capability by using JavaScript code and "cookies." Cookies are browser files maintained on the user's hard drive that store information that normally would vanish when the user moved to another web page. None of the literature web pages has more than 30 KB of data since it was found that data strings larger than 32 KB caused browsers to incompletely load the page. To update the list of citations, the entire database must be reformatted by the BASIC program into 30-KB blocks such that no first author is split between pages. The BASIC applications were programmed with Microsoft QuickBASIC version 4.5, while the Java applet (small application) was done with the Sun Microsystem' 1.2.2 package.

Still another feature is a button ("VP") that displays a window with a JavaScript coded calculator of vapor pressures of a few unsaturated acetate esters as well as saturated aldehydes, alcohols, hydrocarbons, and fatty acids with 10 or more carbon chain lengths (Figure 3). A compound is selected from a pop-up menu on the left and a temperature is entered on the right so that the vapor

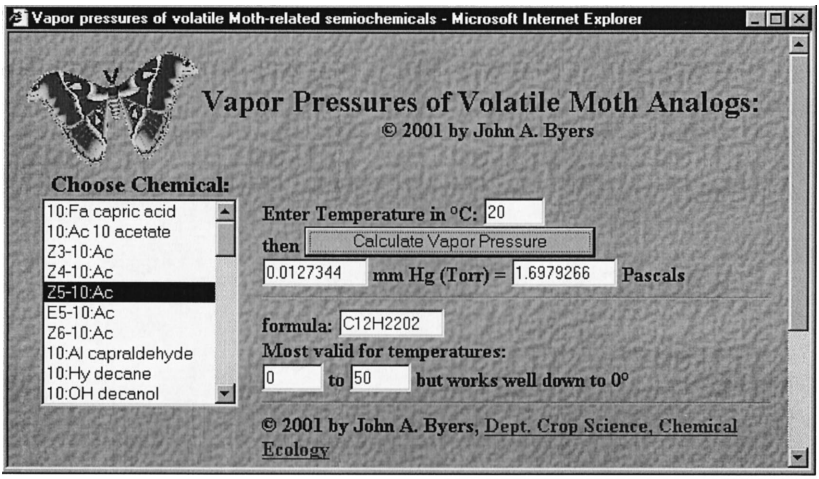

FIG. 3. Internet browser view of a pop-up window of a JavaScript web page for calculating vapor pressures of some moth pheromone components and fatty acids at different temperatures.

pressure (V) can be calculated from the equation, $V = 10^{(-0.2185\,A/K)+B}$, where V is in millimeters of mercury (torrs), A is molar heat of vaporization in calories per gram mole, K is temperature in degrees Kelvin, and B is a constant (Schlessinger, 1971). Exponential regression was used on the data of Olsson et al. (1983) to obtain vapor pressure equations for some moth acetate esters. Vapor pressures can also be estimated from boiling points at partial pressures according to the methods of Hass and Newton (1971).

RESULTS AND DISCUSSION

Operation of the web page for drawing unsaturated straight-chain hydrocarbons with various functional groups is user-friendly. Only two drop-down menu bars and possibly a few checkboxes need to be selected to draw and name the majority of natural moth pheromone components (Figure 1). The program can draw the 43 unbranched hydrocarbons with no functional group, 71 epoxides, 82 aldehydes, 66 alcohols, 167 acetate esters, and 10 other esters listed in the Pherolist. Thus, about 83% of the 528 currently listed components (produced or attractive) in the Pherolist can be drawn (16 additional analogs containing chlorine, fluorine, or nitrogen are considered unnatural). During the extraction of compound names from the Pherolist, names with "methyl," "chlor," "fluor," "one," "oate," "yn," "2-ol," "iso," "nitr," and "valer" were excluded to simplify the drawing function. Of the

71 epoxides known, all but one have one epoxide per compound and these are all *cis*-configured, being either of two chiral forms, *R,S* and *S,R*, so it was possible to include these components (Millar, 2000; Pherolist). However, the chiralities of about half of the aliphatic epoxides (all *cis*) have not been determined. Although chemists have made *trans*-configured epoxides, they have not been discovered in moths (Millar, 2000; Pherolist). One important group that was excluded contains 48 methyl-branched analogs. This group was not considered since the structures are often chiral and, thus, naming becomes more complex. The remaining 49 excluded names were: "one" (16), "oate" (13), "2-ol" (8), "iso" (6), and "valer" (1).

The program also can draw several million unused or undiscovered analogs. For example, considering 14-carbon acetate esters, there are 23 different singly unsaturated compounds, 200 doubly unsaturated ones, and 816 triply unsaturated ones (*E*, *Z*, or delta) (Byers, unpublished). Even more compounds are possible when four (1568), five (1232), and the maximum of six unsaturations (256) are considered. However, further unsaturation in a molecule would make it less stable due to chemical alteration by hydrogenation or geometric isomerization. Natural selection would not favor the evolution of a less stable pheromone component that otherwise would require continuous production of large quantities to be effective. Naturally occurring longer chain hydrocarbons have been reported with up to three unsaturations and rarely four, e.g., (*Z,Z,Z,Z*)-3,6,9,11-nonadecatetraene in *Alsophila pometaria* (Geometridae) (Wong et al., 1984) and (*Z,Z,Z,Z*)-7,13, 16,19-docosatetraen-1-ol isobutyrate in *Euproctis chrysorrhoea* (Lymantiidae) (Leonhardt et al., 1991), but none have been found with five. Theoretically, 10 unsaturations can fit within a 22-carbon chain acetate ester and be named by using the program. An analysis of the 294 unbranched naturally occurring aldehydes, acetate esters, and alcohols (Pherolist) shows that many possible permutations of unsaturations have not been reported in the Lepidoptera. There were 125 components with one unsaturation (42%) and 130 components with two unsaturations (44%). Of the latter, 77% have one single bond in between the unsaturations (conjugated), while 5% have two single bonds between the unsaturations, and 18% have more than two single bonds between. Only 16 components (5%) have three unsaturations and none have four unsaturations (only a few straight chain hydrocarbons have four).

The number of different components reported in moths to date (about 528) appears strikingly few when compared to the several million unsaturated *E* and *Z* analogs potentially available as sex pheromone components. For example, there are 1039 possible 14-carbon chain acetate ester components with one, two, or three unsaturations (*E*, *Z*, or delta) (Byers, unpublished data), but only 42 of these have been identified in moths (Pherolist; Byers, unpublished data). The limitation to essentially 1039 possible analogs may indicate this is a sufficiently large number to allow any species to evolve a unique blend from among these. However, there may not have been enough evolutionary time for speciation events to create more

divergence and use of more structures. The limitation to three or less unsaturations in components may also be due to chemical constraints such as increased instability with desaturation.

The enzyme systems for desaturation and chain-shortening involved in moth component biosynthesis (Bjostad et al., 1981; Roelofs and Wolf, 1988; Tillman et al., 1999) may have evolved in response to the inherent stability of mono- and di-unsaturated hydrocarbons as compared to less stable multiunsaturated alternatives. Probably the most obvious hypothesis to account for the relatively few unsaturations in a moth component is that the fatty acid precursors (oleic, linoleic, and linolenic acids) have one, two, or three unsaturations, respectively. However, as mentioned above, a few moths biosynthesize aliphatic chains with four unsaturations, and many odd- and even-numbered carbon bond positions can be unsaturated by the Δ5, 9, 10, 11, 12, and 14 desaturases and chain-shortening enzymes that have been discovered so far (Bjostad et al., 1981; Roelofs and Wolf, 1988; Foster and Roelofs, 1988, 1996; Zhao et al., 1990; Jurenka, 1997; Tillman et al., 1999). If there are constraints on the evolution of biosynthetic enzymes, there could also be limits on the ability of antennal receptors to discriminate among higher numbers of unsaturated bonds (and constraints on the evolution of more complexity).

The limited numbers of unsaturated positions in moth analogs might also be due to some disadvantage in using molecules with increased volatility due to higher numbers of unsaturated bonds. The double bond length between carbons is shorter than in a single bond (also the H—C bonds are shorter on double bond carbons), which makes the molecule smaller in area and, thus, more volatile (Morrison and Boyd, 1973). There are few data on vapor pressures of moth acetate esters with multiple unsaturations. Olsson et al. (1983) found that *Z*9–14:Ac (16 carbons) has a vapor pressure of 7*E*-4 mm Hg at 30.5°C, which is similar to 14-carbon myristic acid [3*E*-4 mm (Schlessinger, 1971)]. Thus, comparing 18-carbon fatty acids as models for 18:Ac, the differences in vapor pressures among 18-carbon fatty acids with none, one, two, or three unsaturations (stearic, oleic, linoleic, and linolenic acid) appear to be only about 2.5-fold using methods of Schlessinger (1971) and Hass and Newton (1971). Moth species have used pheromone components commonly with chain lengths from 18 down to 10, representing a decrease in volatility for saturated fatty acids of about 54-fold, for alcohols of about 33-fold, and for aldehydes of about 122-fold (Schlessinger, 1971; Hass and Newton, 1971). It seems doubtful that the modest increases in volatility by increasing desaturation would prevent evolution toward use of components with further unsaturation. However, no data were found to ascertain the effect of adding more than three double bonds on vapor pressures of moth pheromone components.

Ultimately, there is no selection pressure or fitness benefit to making a pheromone component more complex (more unsaturations or groups) once a unique species-specific structure has evolved. It is, however, apparently easier to combine two simpler components into a unique blend than to increase the

structural complexity of a single component pheromone to achieve specificity. It is expected that there would be costs to maintain unnecessarily complex systems for biosynthesis and reception when simpler ones suffice.

In moth pheromone components, two unsaturated bonds are always separated by at least one saturated bond, and position 1 is never unsaturated in aldehydes, alcohols, and acetate esters due to a maximum of four carbon bonds, keto–enol tautomerism, and dipole moments, respectively (Morrison and Boyd, 1973). Of the different moth 14-carbon acetate esters with two double bonds, the average number of saturated bonds separating two unsaturated bonds is 1.7 ± 0.66 (95% CL, $N = 20$, Pherolist). Theoretically, if two unsaturated bonds in a 14-carbon chain acetate ester are located in all possible ways such that they are separated by at least one single bond, then the average number of single bonds separating unsaturated bonds would be four (calculated from BASIC code: FOR W = 10 TO 1 STEP -1: FOR X = 1 TO W: C = C + X: D = D + 1: NEXT: NEXT: AVE = C/D). This expected average separation distance of four single bonds is much larger than the observed 1.7 bonds. Of the twenty 14:Ac components, 15 have one bond separation (conjugated), three have more than two bonds, and only one has two bonds between unsaturations (as does the fatty acid 9,12-linoleic acid).

It is also of interest to note that pheromone components and analogs have been identified from relatively few moth species (1683 species from 50 families, Pherolist). In North America alone there are over 65 moth families (Borror and DeLong, 1971), and the number of species worldwide is estimated to approach 185,000 (Holloway et al., 1987). The limited number of species with identified pheromone components is probably because most work has focused on pests, and these species are a small fraction. Secondly, once certain components are known, their subsequent discovery in other species is more likely because standards are more easily obtained and analytical methods for isolation and identification have already been developed. For example, an unknown exotic structure might not pass through the gas chromatographic columns in use, might occur in trace amounts, or have no comparable GC-MS standards (for identification) or commercial sources (for field bioassay).

The web site, linked closely with the Pherolist and reference database, should provide an educational experience for learning about moth pheromone components and aid in research. If a drawn pheromone component is on the Pherolist and the “Pherolist” button is clicked, a pop-up window links automatically to the compound’s web page in the Pherolist. Another button (“Index”) pops up a window displaying the main index of compounds of the Pherolist. As mentioned earlier, the button “Refs” opens a window of a database of over 5900 citations on lepidopteran semiochemicals, and this page links to several other citation databases on semiochemicals of Coleoptera (2256 citations), Hymenoptera (1724 citations), and other insects (2348 citations). The vapor pressure calculator (“VP” button) can aid in predicting appropriate release rates of components in the lab and field

in ratios depending on the mole percent in solution times the vapor pressure. For example, if components A:B have a mole percent ratio of 1:10 in solution and vapor pressure ratios of 1:100, then component B will have a 1000-fold higher release rate than A.

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