

STIMULATION OF TARSAL RECEPTORS OF THE BLOWFLY BY ALIPHATIC ALDEHYDES AND KETONES*

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(Received for publication, September 30, 1948)

In an effort to define the relationship between the chemical and physical properties of compounds and their effectiveness as stimuli for chemoreception, the response of blowflies (*Phormia regina* Meigen) to tarsal contact with aqueous solutions has now been studied in several different aliphatic series. Results obtained with the primary normal alcohols and with several types of glycols (2, 3) showed in each case a logarithmic increase in stimulative potency as the carbon chain was lengthened. Straight carbon chains proved to be more stimulating than isomeric branched chains or than chains containing ether linkages, and the simple alcohols were more effective than the corresponding dihydroxy compounds, a finding which agreed with results obtained earlier with various aliphatic acids (1). With the objective of gaining additional information as to the fundamental factors in sensory stimulation, we have in the present study extended this type of observation to two additional series, similar in chemical structure to those previously examined but with different polar groups.

Materials and Methods

Details of the experimental technique and of the method of treating the data have already been described (2, 3). Compounds used in the tests reported below included aldehydes, ketones, and a series of alcohols comparable to the latter in the position of the polar group. The following grades of chemicals were available:

Methanal.....	formaldehyde Merck reagent
Ethanal.....	acetaldehyde Eastman B. P. 20–22°C.
Propanal.....	propionaldehyde Eastman B. P. 47–49°C.
Butanal.....	<i>n</i> -butyraldehyde Eastman B. P. 72–74°C.
Heptanal.....	<i>n</i> -heptaldehyde Eastman B. P. 40–42°C./10 mm.
2-Ethylhexanal.....	octaldehyde Carbon and Carbide Chemicals Corp. B. P. 163.4°C.
2-Methylpropanal.....	<i>iso</i> -butyraldehyde Eastman B. P. 63–64°C.

* The work described in this paper was done under contract between the Medical Division, Chemical Corps, United States Army and The Johns Hopkins University. Under the terms of this contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the authors.

3-Methylbutanal.....	<i>iso</i> -valeraldehyde Eastman	B. P. 91-93°C.
2-Propanone.....	acetone Merck reagent	
2-Butanone.....	methyl ethyl ketone Eastman	B. P. 79-80°C.
2-Pentanone.....	methyl <i>n</i> -propyl ketone Phillips Scientific Specialties Co.	
2-Heptanone.....	methyl <i>n</i> -amyl ketone Eastman	B. P. 149- 150°C.
3-Pentanone.....	diethyl ketone Eastman	B. P. 100.5-102.5°C.
4-Heptanone.....	di- <i>n</i> -propyl ketone Phillips Scientific Special- ties Co. (Technical grade).	
5-Nonanone.....	di- <i>n</i> -butyl ketone Eastman	B. P. 188.5- 192.5°C.
3-Pentanone, 2,4-dimethyl-.....	di- <i>iso</i> -propyl ketone Eastman (Pract.)	
2-Propanol.....	<i>iso</i> -propyl alcohol Eastman	98-99 per cent
2-Butanol.....	<i>sec</i> -butyl alcohol Eastman	B. P. 99-101°C.
2-Pentanol.....	<i>sec-act</i> -amyl alcohol Eastman	
2-Heptanol.....	methyl- <i>n</i> -amyl carbinol Paragon Testing Laboratories	
2-Octanol.....	capryl alcohol Merck	
3-Pentanol.....	diethyl carbinol Eastman	B. P. 114-116°C.

RESULTS

In Table I are collected the data required for a comparison of the compounds and for defining the response of the blowfly population to each.

DISCUSSION

Five normal aldehydes, two *iso*-aldehydes, and 2-ethylhexanal were tested. As shown in Fig. 1, the plot of rejection thresholds against chain length is similar in nearly all respects to that found previously for the normal alcohols, and includes a rather sharp break or change in slope between the three and four carbon members. The upper limb of the curve is fitted by a line having the equation

$$Y = 0.0690 - 1.2010(X - 0.2593)$$

(variance of $a = 0.0104$; variance of $b = 0.0232$)

and the lower limb (2-ethylhexanal excluded) by

$$Y = 1.9737 - 11.2533(X - 0.7153)$$

(variance of $a = 0.0176$; variance of $b = 1.2567$).

Although the slopes for these lines are not significantly different from those for the corresponding portions of the curve for normal alcohols (the difference in slope divided by its standard error is less than 3 in both cases), the position of the aldehyde curve is lower on the graph and the individual aldehydes were more stimulating than the corresponding alcohols in all of the seven pairs tested. They averaged about 2.66 times as effective.

Eight sufficiently water-soluble ketones were available. Since this small group includes representatives of several structurally different series and since some of the threshold values appear slightly aberrant, lines of best fit have not been determined. However, the ketones clearly follow the same sort of

TABLE I
Response of Phormia to Aldehydes, Ketones, and Secondary Alcohols in 0.1 M Sucrose

Compound	No. of C atoms	Log molar concentration rejected by 50 per cent ± 2.575 S.E.	$a \pm \text{S.E.}^*$	$b \pm \text{S.E.}^*$	\bar{x}	No. of flies tested
Methanal	1	0.365 \pm 0.135	5.124 \pm 0.144	2.766 \pm 0.381	0.410	101
Ethanal	2	0.061 \pm 0.147	4.467 \pm 0.131	2.610 \pm 0.350	-0.143	150
Propanal	3	-0.219 \pm 0.120	4.952 \pm 0.184	3.952 \pm 0.718	-0.231	100
Butanal	4	-0.837 \pm 0.147	4.858 \pm 0.154	2.727 \pm 0.440	-0.889	100
Heptanal	7	-3.531 \pm 0.166	4.715 \pm 0.145	2.371 \pm 0.386	-3.651	100
2-Ethylhexanal	8	-3.688 \pm 0.148	4.917 \pm 0.155	2.709 \pm 0.443	-3.718	100
2-Methylpropanal	4	-0.717 \pm 0.152	5.159 \pm 0.136	2.339 \pm 0.336	-0.649	100
3-Methylbutanal	5	-1.553 \pm 0.189	5.251 \pm 0.173	2.449 \pm 0.479	-1.450	75
2-Propanone	3	-0.094 \pm 0.257	5.100 \pm 0.200	2.022 \pm 0.508	-0.044	135
2-Butanone	4	-0.597 \pm 0.136	5.136 \pm 0.133	2.540 \pm 0.297	-0.544	120
2-Pentanone	5	-1.220 \pm 0.162	5.010 \pm 0.132	2.108 \pm 0.277	-1.225	140
2-Heptanone	7	-2.529 \pm 0.208	4.870 \pm 0.208	2.600 \pm 0.552	-2.579	105
3-Pentanone	5	-1.274 \pm 0.145	5.183 \pm 0.125	2.258 \pm 0.310	-1.194	158
4-Heptanone	7	-2.855 \pm 0.185	5.244 \pm 0.156	2.789 \pm 0.451	-2.768	100
5-Nonanone	9	-2.770 \pm 0.171	4.900 \pm 0.151	2.285 \pm 0.418	-2.814	100
3-Pentanone, 2,4-dimethyl-	7	-2.022 \pm 0.150	5.015 \pm 0.144	2.471 \pm 0.377	-2.020	100
2-Propanol	3	0.272 \pm 0.036	5.244 \pm 0.194	10.239 \pm 1.725	0.296	105
2-Butanol	4	-0.011 \pm 0.029	5.225 \pm 0.154	11.300 \pm 1.415	0.009	100
2-Pentanol	5	-0.856 \pm 0.084	5.104 \pm 0.155	4.753 \pm 0.647	-0.834	124
2-Heptanol	7	-2.833 \pm 0.161	5.008 \pm 0.150	2.350 \pm 0.394	-2.823	100
2-Octanol	8	-2.866 \pm 0.193	4.950 \pm 0.328	3.213 \pm 0.766	-2.881	50
3-Pentanol	5	-0.908 \pm 0.105	5.222 \pm 0.111	2.801 \pm 0.359	-0.829	160

S.E. = standard error.

* The 4th, 5th, and 6th columns of the table give the calculated values for a , b , and \bar{x} in the equation $Y = a + b(X - \bar{x})$, which is the regression of per cent flies rejecting, Y , expressed as probits, on log concentration, X .

relationship observed in other series and seem in general to be intermediate in stimulating effect between the corresponding alcohols and the aldehydes. That the ketones should be somewhat less effective than the aldehydes would be expected from the position of the polar group, which is terminal in the

aldehydes but subterminal in the ketones. As noted previously, alcohols with the hydroxy substitution elsewhere than in the 1-position are slightly less stimulating than their normal isomers (3). This is seen also in Fig. 1, where threshold values for five secondary alcohols and 3-pentanol may be compared both with the lines of best fit for the normal series and with the individual values for the corresponding ketones.

Altogether, the differences between any of the series, though fairly consistent,

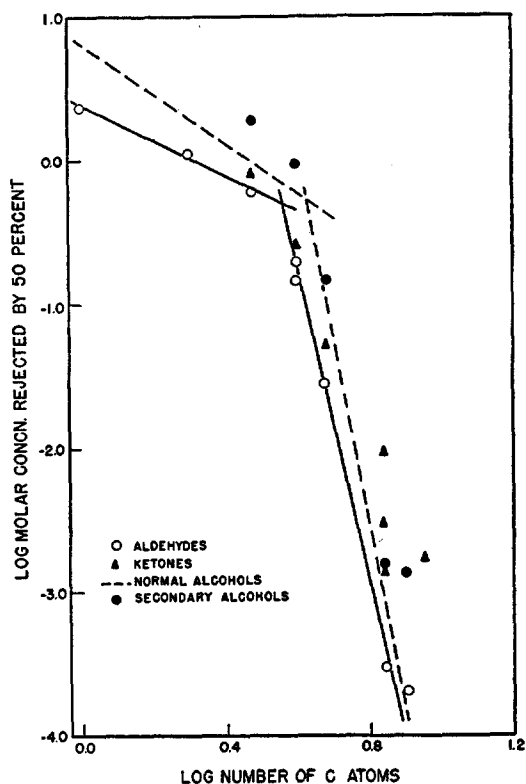


FIG. 1. Rejection of aldehydes, ketones, and secondary alcohols by *Phormia*.

are not great. It seems, therefore, that while the nature and position of the polar group are of some importance in determining the stimulating effect, the length of the carbon skeleton is a more significant factor for series of this sort.

The data now at hand for alcohols, glycols, aldehydes, and ketones suggest three principal questions: (1) Why is there regularly an increasing effectiveness of the members of each series as chain length is increased? (2) Why is the rate of increase in stimulating effect with increasing chain length less among the lower than among the higher members of a single series? (3) What chemical

or physical attributes determine the order of effectiveness of the several series studied, which is in descending order: aldehydes, ketones, alcohols, glycols?

Despite the fact that there is within each series a very high degree of correlation between the observed threshold values and boiling points or other properties related thermodynamically to them and to chain length, our attempts to find a single factor or set of factors which would reconcile the results from different series and from two portions of each series have been largely unsuccessful. The task has been made more difficult by the scarcity of pertinent physical data, for, with the exceptions of boiling and melting points, the values available for the various properties which might be significant are relatively incomplete, and in most cases a rigorous analysis has not been possible. Again, many of the properties themselves vary logarithmically with chain length and therefore yield correlations of the sort shown in Fig. 1, which does not bring the solution of the problem any nearer. Among these may be mentioned molecular weights, molecular volumes and areas, oil-water distribution coefficients, activity coefficients, standard free energies, vapor pressures, and boiling points.

Other objections to regarding the boiling point as an expression of the forces basically concerned in stimulation are the following: within a given series an increase in stimulating power accompanies an increase in boiling point, whereas the reverse is true when the several series are compared with one another. Thus, in the range of lower molecular weights, the order of increasing boiling points is: aldehydes, ketones, alcohols, diols: but this is the order of decreasing effectiveness in stimulation. Yet, among the various types of monohydric alcohols, boiling point and stimulating power both increase in the direction: secondary, *iso*-, primary. In view of contradictions such as these it seems certain that the factors involved in stimulation are not identical with those which determine the boiling point.

Dipole moments from the study by Smyth (8) gave an excellent correlation with threshold values in the series of normal alcohols (2). Possibly this was fortuitous, inasmuch as other determinations of dipole moments within a given series do not show a regular progression, and in fact vary but little (*cf.* reference 6). In any case, the differences between series cannot be explained on the basis of dipole moments, for the latter increase in passing from alcohols to aldehydes to ketones (4-6), and this is not the order of relative stimulating power. A plot of the threshold data against dielectric constants also was not particularly helpful, partly because of variation in the value for this property under differing conditions of measurement and the absence of standard conditions of measurement in many of the determinations reported.

Since it is known that the degree of association of polar-non-polar aliphatic compounds is greater in the lower range of molecular weights (9), it seemed possible that this might be concerned in the change in slope observed in plots of threshold values against chain length. Reasoning that the increase in boiling

point of the alkanes upon substitution of a polar group is in some degree a measure of the increased tendency for association (*cf.* reference 7), we compared the threshold values for the normal alcohols and aldehydes with the differences in boiling point between these compounds and the corresponding normal alkanes. However, this procedure failed to eliminate the change in slope. One may argue also that association would decrease the effective molarity of the test solutions; correcting for this, if possible, would merely decrease the observed threshold molarities more for the lower members of a series and would thus accentuate the difference in slope. For these reasons, it seems doubtful that degree of association is a factor of much importance in the observations.

We have been unable, then, to bring our data into a single homogeneous system through correlations with the following molecular properties: number of carbon atoms, molecular weights, molecular areas and volumes, oil-water distribution coefficients, activity coefficients, standard free energies, vapor pressures, boiling points, melting points, dipole moments, dielectric constants, and degree of association. Thus we must conclude either that we have overlooked the significant factors or that we are not dealing with a single process throughout, since the evidence from the series considered individually is convincing that stimulation is in some way related to the thermodynamic properties of the compounds.

One other comparison remains to be discussed. This is between the threshold concentrations of the compounds and their solubilities in water. Here again a quantitative evaluation is impossible, because of the incompleteness of published solubility data and because we have no means of grading the solubility among compounds which are freely miscible with water. It is a fact, however, that the order of stimulative effectiveness follows the inverse of the order of water solubilities with fewer contradictions than appear in most of the other comparisons attempted. Thus, within each series, solubility decreases consistently as chain length and stimulating power increase. The order of decreasing solubility: diols, alcohols, aldehydes is also the order of increasing stimulating effect, and *iso*-aldehydes are more soluble and less stimulating than the normal compounds, while the order of decreasing solubility for the isomeric alcohols: secondary, *iso*-, normal, is likewise that of increasing stimulation. 2-Butanone, on the other hand, is more stimulating than 2-butanol and slightly more soluble, so that it constitutes an exception; among the higher members compared in these two series both solubilities and threshold values tend to agree closely.

In spite of the generally good correspondence between low solubility in water and high stimulating power, it seems likely, from the data on oil-water distribution coefficients, that plots of threshold values against solubilities would also yield a smaller slope for the lower than for the higher range of compounds

in each series, if such an analysis could be made. The consistent recurrence of this type of relationship, which is well attested for the aldehydes, primary alcohols, and diols, strongly indicated for the ketones, *iso*-alcohols, and secondary alcohols (series lacking the one and two carbon members), and which seems to have no counterpart in any of the tabulated values for the physical properties, prompts us to consider the possibility that different forces may be of primary importance in stimulation by the lower and higher members of each of the types investigated. This amounts to postulating at least a two-phase system for the limiting mechanism in contact chemoreception. The hypothesis that smaller molecules gain access to the receptors in part through an aqueous phase, while the larger aliphatic molecules penetrate chiefly through (or accumulate in) a lipoid phase, would appear to offer a basis for reconciling most of the contradictions encountered when it is attempted to fit the facts into a single-phase system. Movement of the smaller molecules through an aqueous medium should occur at rates related inversely to the molecular weight, which would help to account for their being more stimulating than anticipated from the relationship found for the higher members of a series, though it is doubtful that the entire difference can be explained in this way. It may be noted also that the inflections in the curves relating thresholds to molecular size occur at increasing chain lengths in passing from the less to the more water-soluble species. At the same time the predominant importance of lipoid affinity is suggested by the logarithmically increasing stimulating power of both lower and higher members of all series, as well as by the inverse relationship between water solubility and stimulating effectiveness in comparisons of the several series with each other. Although it is not known to what extent recent observations on the structure of insect cuticle (10, 11) may apply to the chemoreceptive surface, a scheme such as that proposed here is in accord with current views as to the general makeup of the integument, and would also allow for the fact that highly water-soluble ions as well as nearly insoluble non-electrolytes are adequate stimuli when presented in an aqueous medium.

SUMMARY

Rejection of eight aldehydes, eight ketones, five secondary alcohols, and 3-pentanol has been studied in the blowfly *Phormia regina* Meigen. The data agree with results previously reported for normal alcohols and several series of glycols in showing a logarithmic increase in stimulating effect with increasing chain length. The order of increasing effectiveness among the different species of compounds thus far investigated is the following: polyglycols, diols, secondary alcohols, *iso*-alcohols, normal alcohols, ketones, *iso*-aldehydes, normal aldehydes.

Curves relating the logarithms of threshold concentration to the logarithms of chain length for diols, alcohols, aldehydes, and ketones show inflections in

the 3 to 6 carbon range. Above and below the region of inflection the curves are nearly rectilinear. The slopes for the upper limbs (smaller molecules) are of the order of -2 ; for the lower limbs, about -10 .

Comparisons of the threshold data with numerical values for molecular weights, molecular areas and volumes, oil-water distribution coefficients, activity coefficients, standard free energies, vapor pressures, boiling points, melting points, dipole moments, dielectric constants, and degree of association are discussed briefly, and it is concluded that none of the comparisons serves to bring the data from the several series and from the two portions of each series into a single homogeneous system. A qualitative comparison with water solubilities shows fewer discrepancies.

It is suggested that the existence of a combination of aqueous and lipid phases at the receptor surface would fit best with what is presently known about the relationship between chemical structure and stimulating effect in contact chemoreception. In this hypothesis the smaller and more highly water-soluble compounds are envisaged as gaining access to the receptors partly through the aqueous phase, the larger molecules predominantly through the lipid phase.

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