

den Eischalen von *Natrix natrix* ebenfalls kollagenähnliche Proteine vorkommen, die die Stabilität und hohe Zugfestigkeit dieser Eischalen im Vergleich zu kalkigen Eischalen erklären könnten¹⁵. Diese Eigenschaften werden zweifellos von Quervernetzungen durch Disulfidbrücken unterstützt, wie sie für die Cuticula von *Ascaris*¹⁶ und für die Kapseln von Nematocysten der Seeanemone¹⁷ angenommen worden sind. Die Hohlräume im Faser-Core der Ringelnatter-Eischalen könnten ebenfalls helfen, die mechanischen Eigenschaften zu verbessern; gleichzeitig ist aber auch eine Beteiligung am Gas- und Flüssigkeitsaustausch zu vermuten.

Summary. The ultrastructures of eggshells of *Natrix natrix* and the membrana testacea of *Gallus gallus dom.* are very similar, with the exception of cavity systems which only exist in eggshells of snakes (ringsnake) but not in the membrana testacea of birds. In the sheath of the fibres of *Natrix natrix*, a positive ruthenium red-reaction indicates the presence of mucopolysaccharides, or proteoglycans. The eggshells of *Natrix natrix* contain a high proportion of proline, but also of cystine, histidine

and arginine. The high proline content is discussed with regard to the properties of poly-L-proline. Presumably proteins similar to collagen exist in eggshells of *Natrix natrix*.

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The Molecular Basis for Scent Discrimination: Response to Nitrobenzene-*d*₅ of Honey Bees (*Apis mellifera* L.) Conditioned with Nitrobenzene

The substitution of an isotopic atom such as deuterium for protium in a molecule containing hydrogen should leave essentially unchanged those molecular properties that are associated with electronic structure and force fields. In contrast, molecular properties dependent upon mass are changed¹. If the chemoreceptor system for odor detection involves an electric field that fits a receptor site², the replacement of hydrogen with deuterium should not change the aroma of a molecule. If it involves molecular motions or dipole moments³, the isotopic effects should cause changes.

When 4-(*p*-hydroxyphenyl)-2-butanone acetate was systematically deuterated, substitutions of part of the hydrogen atoms did not affect the potent attractiveness of the compound to males of the melon fly⁴. When the activity of 7 partially deuterated ketones was tested with the harvester ant⁵, the deuteration of active hydrogen atoms adjacent to the carbonyl group did not affect the ant's alarm-releasing activity. In both experiments, part of the IR- and far-IR-absorption peaks related to hydrogen were shifted to lower frequencies with the heavier isotope, shifts that were related to the site of deuteration. No completely deuterated compound has been tested.

The absorption spectra of nitrobenzene was used to support a hypothesis³ that odor determination was

related to out-of-plane deformation of the benzene ring at 397 and 176 cm⁻¹. Other peaks supported speculation that it was not the vibrational energy alone that influenced odor perception. Instead, the effects of matching frequencies³ on the shape of the molecular boundary seemed to influence VAN DER WAAL'S interactions with the receptor surface to cause fine discrimination between odors.

Materials and methods. Nitrobenzene-*d*₅ (mol. wt. 123, b.p. 208° m.p. 6°, [α]_D²⁰ 1.5498, purity 99%) should be even more stable than nitrobenzene to free radical or ionization reactions. Both liquids, as purchased⁶, contained normal traces of impurities that colored the faint yellow. Chromatography on a charcoal column and distillation did not remove all the color.

When nitrobenzene was coated between salt or polyethylene plates and IR-absorption measured with Beckman IR-8 and IR-11 spectrophotometers, the expected peaks were found. Nitrobenzene-*d*₅ had different peaks: m1588, s1514, s1362, s1340, w1298, w1073, w870, m843, w812, w715, and m651. In addition, the peaks at 3080, 701, and 395 cm⁻¹ were missing, and even those

Table I. Responses of honey bees to 1% nitrobenzene vs. 1% benzaldehyde when conditioned to 1% benzaldehyde

Time test began (h)	Time for 50 bees to land (min)	Bees responding to nitrobenzene (%)	Bees responding to benzaldehyde (%)
09.10	3	26	74
10.07	3	26	74
10.45	2.5	28	72
11.30	3	26	74
Mean		26.5	73.5

¹ K. B. WIBERG, *Chem. Revs.* 55, 713 (1955).

² R. W. MONCRIEFF, *The Chemical Senses*, 2nd edn. (Leonard Hill Ltd., London, 1951). - J. E. AMOORE, *Nature, Lond.* 198, 271 (1963); 233, 270 (1971). - Anonymous, *Chem. Eng. News* 23, 30 (1968).

³ G. M. DYSON, *Perfum. essent. Oil Rec. yb.* 28, 13 (1937). - L. H. NARODY, *Perfum. essent. Oil Rec. yb.* 47, 23 (1956) - R. H. WRIGHT, *J. appl. Chem.* 4, 611 (1954); *Nature, Lond.* 198, 455 (1963); *Nature, Lond.* 209, 571 (1966). - R. H. WRIGHT and A. ROBSON, *Nature, Lond.* 222, 290 (1969).

⁴ R. E. DOOLITTLE, M. BEROZA, I. KEISER, E. L. SCHNEIDER, *J. Insect Physiol.* 14, 1697 (1968).

⁵ M. S. BLUM, R. E. DOOLITTLE, M. BEROZA, *J. Insect Physiol.* 17, 2351 (1971).

⁶ Nitrobenzene and nitrobenzene-*d*₅ were purchased from Aldrich Chemical Co., Milwaukee, Wisc. The use of trade names and the listing of suppliers does not constitute recommendation of a particular product by the U.S. Dept. of Agriculture.

Table II. Responses of honey bees to either 1% nitrobenzene vs. Nujol or 1% nitrobenzene- d_5 vs. Nujol when conditioned to 1% nitrobenzene

Time test began (h)	Time for 50 bees to land (min)	Bees responding to nitrobenzene (%)	Bees responding to nitrobenzene- d_5 (%)
09.52	5.5	68	
10.26	5		56
11.15	6	70	
11.52	5.5		64
13.24	6.5	62	
14.10	4		62
14.51	4	48	
15.34	5.5		66
Mean		62	62

Table III. Responses of honey bees to 1% nitrobenzene vs. 1% nitrobenzene- d_5 when conditioned to 1% nitrobenzene

Time test began (h)	Time for 50 bees to land (min)	Bees responding to nitrobenzene (%)	Bees responding to nitrobenzene- d_5 (%)
09.04	4.5	54	46
09.55	4.5	52	48
10.50	5	46	54
11.34	5	42	58
13.02	3.5	54	46
13.41	4.5	54	46
14.30	5	56	44
15.11	8.5	46	54
Mean		51	49

peaks assignable to $-\text{NO}_2$ were shifted by interactions. Thus, aside from some similarity in the peaks assigned to $-\text{NO}_2$ stretching near 1525 and 1350 cm^{-1} , the spectra of nitrobenzene and nitrobenzene- d_5 have no peaks in common.

A bioassay that utilizes honey bees, *Apis mellifera* L.⁷, was employed. Bees were conditioned for 6 h to associate the odor of 1% chemical in mineral oil with the availability of 30% sucrose solution. Test scents were put onto filter paper in each of 6 beakers 5 cm below the aroma ports of a test arena that rotated at $1/3$ rpm. A metal screen was placed over each port and changed after a bee had landed to reduce the probability that bee odor influenced the selection of an aroma port by the bees⁸. The results of at least 4 tests (50 visits per test) were used to calculate a pooled chi square value which determined the statistical significance of our results.

Results. When bees were allowed to choose between 1% nitrobenzene and 1% benzaldehyde, both odors described⁹ as 'bitter almond', the results (Table I) showed conclusively that bees distinguished between these 2 similar compounds with 73.5% of the bees responding to benzaldehyde ($P < 0.01$).

When bees were conditioned to nitrobenzene and were allowed to choose between nitrobenzene vs. control or nitrobenzene- d_5 vs. control, the higher numbers of bees visiting scented beakers (Table II) were highly significant ($P < 0.01$). Although nitrobenzene vapor can be poisonous to some animals and benzaldehyde is sometimes used as a bee repellent, the conditioned bees did use the aromas to find sugar solution. The minutes elapsed in each test before 50 bees landed did not indicate any delay in response to the deuterated compound as compared with the undeuterated. Thus, bees conditioned to nitrobenzene responded identically to both isotopic analogs.

In a more critical comparison the bees chose between 3 beakers that contained 1% nitrobenzene and 3 that contained 1% nitrobenzene- d_5 . The results further support the contention that conditioned bees did not distinguish nitrobenzene from nitrobenzene- d_5 (Table III).

Discussion. Present indications are that the receptor cells for olfaction in honey bees, like those in melon flies and ants, do not allow the insect to distinguish isotopic analogs of certain odorous chemicals. Such findings augment other results based on odor differences in enantiomers⁹ to support a concept that electronic structure and force fields are involved in odor discrimination.

Zusammenfassung. Honigbienen, *Apis mellifera* L., wurden dressiert, Zuckersirup an Stellen zu sammeln die mit Nitrobenzol markiert waren. Wenn Nitrobenzol oder Nitrobenzol- d_5 zur Auswahl standen, konnten die Bienen nicht zwischen diesen Analogen unterscheiden. Dieses Ergebnis stimmt mit der Überlegung überein, dass die Chemorezeptormechanismen mit Elektronenmustern oder -effekten zusammenhängen und nicht auf Oszillations- und Rotationsfrequenz, Dipolmoment oder anderen Masseneffekten an Molekülen beruhen.

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Plasma Pre- α -lipoproteins in Ethionine Induced Fatty Liver in Rats

It is well known that ethionine interferes with the synthesis of β -, pre- β - and α -lipoprotein in the liver¹⁻³. No effect of ethionine on pre- α -lipoprotein, however, has been reported. In the present communication a very specific effect on pre- α -lipoprotein synthesis in the liver by ethionine treatment is reported.

Female rats of Wistar strain, having a mean weight of 200 g, were used. All rats were fed on a diet rich in carbohydrates for 24 h together with drinking water enriched with 20% D-glucose. The animals were then fasted for 24 h and given ethionine injections i.p., 4 injections of 50 mg each². The fasting control rats received 0.9% NaCl