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Enantioselective Syntheses of Georgyone, Arborone, and Structural Relatives. Relevance to the Molecular-Level Understanding of Olfaction

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Abstract: Georgyone (**1**) and arborone (**2**), powerful woody odorants, have been synthesized enantioselectively along with their enantiomers. Several structural relatives of **1** and **2** have also been made enantioselectivity in order to probe the molecular details of the binding of **1** and **2** to the olfactory G-protein-coupled receptors which they activate. These studies have led to a number of conclusions regarding the structural requirements for woody odor, including absolute configuration, critical methyl substitution, and the spatial orientation of the key methyl groups. Odorants **1** and **2** bind to at least 10 mouse olfactory receptors, lending support to the combinatorial model for odor perception/differentiation. The implications of this work with regard to possible receptor binding modes are discussed.

Humans can distinguish many thousands of odorants using about 340 different olfactory receptors (ORs) falling into an even smaller number of olfactory receptor families (ORFs, ca. 70). Exactly how this comes about and the biochemical/physiological mechanisms of olfactory signal transduction/modulation/processing presents an interesting challenge to scientists in several disciplines, including chemical biology. The problem is intriguing from a chemical biology viewpoint because of recent advances in our understanding of the olfactory system which reveal a surprisingly straightforward organization.[1]

Each of the 340 unique ORs is expressed by a unique olfactory neuronal cell type (ON) to which it is connected. The ORs reside on ciliae that project into the olfactory epithelium (an area of ca. 5 cm^2 in the human nose), where they receive and bind odorants.[1] ORs are G-protein-coupled receptors with seven helical transmembrane domains. Although any given type of OR appears to be irregularly and widely distributed over the sensor area, all ONs of the same type are axonally connected to a single neuron-type-specific glomeruler cell in the olfactory bulb. Each glomeruler cell in the olfactory bulb is connected via its specific mitral cell to the olfactory region of the brain.[1,2] Each type of glomerulus occupies a specific location in the olfactory bulb, and the spatial arrangement is the same in different individuals. The arrangement in one hemisphere of the olfactory bulb is duplicated in the other with bilateral symmetry. The details of how all the information is encoded and processed locally in the glomeruli and mitral cells of the olfactory bulb for transmission to the brain are not known.[1−4]

Our interest in the problem of how humans are able to distinguish among so many odiferous molecules and the availability to us of powerful new methods of synthesis of a class of commercial odorant molecules with diastereo- and enantio-control motivated the studies reported herein. It is well known that stereochemistry and absolute configuration are of crucial importance in determining human perception of odors.[5] In addition, molecular size, shape, and functionality are all important determining factors. A number of interesting questions arise which might be answered by chemical/biological studies. For example: (1) Do receptors recognize functional groups in ligands by interactions other than van der Waals forces or H-bonding? (2) Are olfactory receptors sufficiently conformationally flexible to allow a series of odorant ligands of varying structure to bind in more than one mode with a particular OR? (3) If there is such variability in ligand binding to an OR, can this affect the signal emanating from that OR with different ligands? (4) Could G-protein signaling for a particular OR be complicated by the availability of multiple ligand binding sites? (5) How do ORs distinguish very small changes in ligand structure, such as changing a methyl group to a hydrogen?

(1) (a) Axel, R. Nobel Lecture in Physiology and Medicine for 2004. *Angew. Chem., Int. Ed.* **2005**, *44*, 6111−6127. (b) Buck, L. B. Nobel Lecture in Physiology and Medicine for 2004. *Angew. Chem., Int. Ed*. **2005**, *44*, 6128−6140 and www.nobel.se. (c) Buck, L. B. *Annu. Rev. Neurosci*. **1996**, *19*, 517−544.

(2) (a) Buck, L. B.; Axel, R. *Cell* **1991**, *65*, 175−187. (b) Malnic, B.; Godfrey, P. A.; Buck, L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2584−2589. (c) Godfrey, P. A.; Malnic, B.; Buck, L. B. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2156−2161. (d) Malnic, B.; Hirono, J.; Sato, T.; Buck, L. B. *Cell* **1999**, *96*, 713−723. (e) Buck, L. B. *Cell* **2000**, *100*, 611−618. (f) Takahashi, Y. K.; Kurosaki, M.; Hirono, S.; Mori, K. *J. Neurophysiol.* **2004**, *92*, 2413−2427. (g) Uchida, N.; Takahashi, Y. K.; Tanifuji, M.; Mori, K. *Nature Neurosci.* **2000**, *3*, 1035−1043. (h) Ronnett, G. V.; Moon, C. *Annu. Rev. Physiol.* **2002**, *64*, 189−222. (i) Vosshall, L. B. *Curr. Opin. Neurobiol*. **2000**, *10*, 498−503.

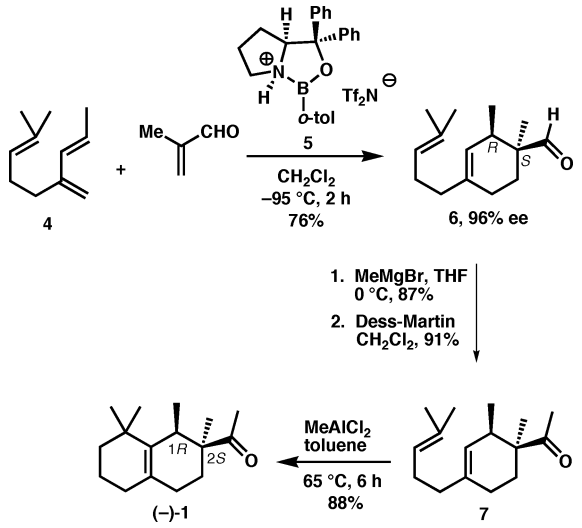
(3) Glusman, G.; Yanai, I.; Lancet, D. *Genome Res*. **2001**, *11*, 685−702 (the complete human olfactory subgenome).

(4) Serizawa, S.; Miyamichi, K.; Nakatani, H.; Suzuki, M.; Saito, M.; Yoshihara, Y.; Sakano, H. *Science* **2003**, *302*, 2088−2091 (regulation of OR gene expression).

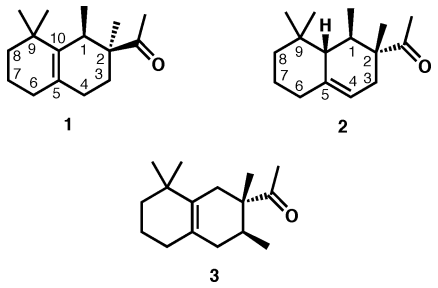
(5) (a) Pybus, D. H.; Sell, C. S. *The Chemistry of Odors*; RSC Paperbacks, Royal Society of Chemistry: London, 1999. (b) Kraft, P.; Bajgrowicz, J. A.; Denis, C.; Fráter, G. *Angew. Chem., Int. Ed.* **2000**, *39*, 2980−3010; see also http://www.iff.com/Ingredients.nsf. (c) Brenna, E.; Fuganti, C.; Serra, S. *Tetrahedron: Asymmetry* **2003**, *14*, 1−42.

10.1021/ja057483x CCC: $33.50

Scheme 1



To address such questions, we started with the odorant substance known commercially as "Georgywood", a racemic mixture of **1** and its enantiomer.[5b] Georgywood possesses a characteristic pleasant woody odor, different from those of cedar-wood and sandalwood, which exhibit distinctive and characteristic odor notes in addition to a "woody" scent.[5−7] An even more widely acclaimed woody odorant than Georgywood is the product "Iso E Super", which is a mixture of several isomeric racemic compounds containing <5% of **2** and its enantiomer and 60% of **3** and its enantiomer.[5b] Remarkably,



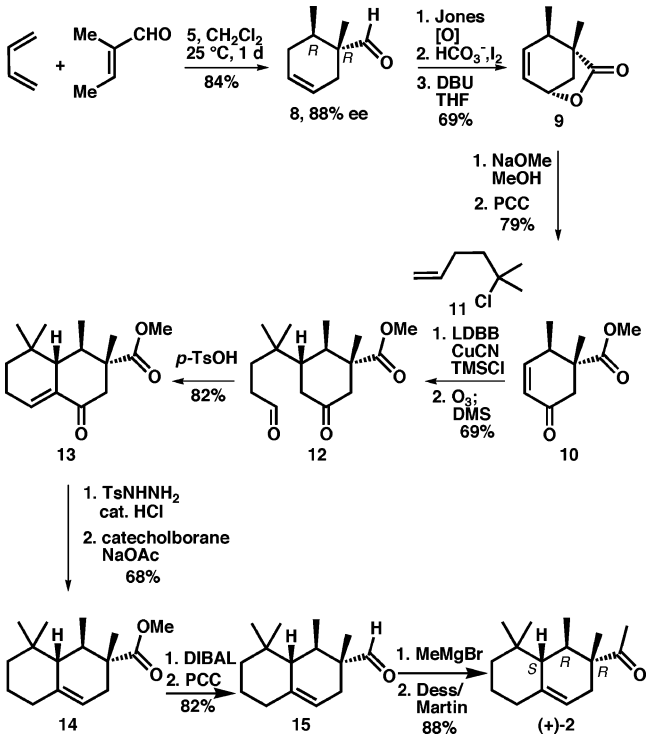
the highly desirable rich warm-woody odor of Iso E Super is due to the minor racemic component (±)-**2**, since (±)-**3** has a threshold odorant concentration 10^5 times greater.[5b,8] Our research started with the development of enantioselective syntheses of chiral **1** and **2** and their enantiomers. The enantiomeric forms of **1** and **2** had not previously been synthesized. One of the first objectives of this work was to identify the exact stereostructure of the effective odorants of Georgywood[7] and Iso E Super.[8]

The enantioselective total synthesis of **1** was accomplished by the sequence that is outlined in Scheme 1. The key step in this process is the enantioselective Diels−Alder reaction of diene **4** with 2-methylacrolein, catalyzed by the (*S*)-oxazaborolidinium salt **5**.[9] As expected from previous studies, this reaction was highly enantioselective and produced the adduct **6** in 96% ee and 76% yield, together with 12% of the diastereomeric (1*S*,2*S*)-adduct (from an exo [2+4] pathway).[10] The assignment of stereochemistry to **6** follows from previous work.[9] The adduct **6** was transformed efficiently into (−)-**1**, the (1*R*,2*S*)-enantiomer of Georgywood, in three straightforward steps. The dextrorotatory (1*S*,2*R*)-enantiomer of **1** was synthesized by the corresponding pathway using the (*R*)-enantiomer of catalyst **5** (*ent*-**5**) for the Diels−Alder step. Whereas **1** possesses an intense clean woody odor, the (+)-enantiomer was found to possess a relatively weak odor which is best described as distinctly unpleasant-acrid-musty.[11] It is fortunate that the pleasant odor of **1** masks the disagreeable odor of *ent*-**1** in the commercial scent. After our assignment of absolute configuration to the active odorant enantiomer of Georgywood was transmitted to the Givaudan group (July 16, 2004), it was accepted by them as consistent with rotation data obtained with a sample of **1** that had been prepared by resolution of a racemic intermediate.[12] They also reported that the threshold for odor detection of *ent*-**1** is 10^3 times greater than that for **1**. We believe that the synthesis of **1** outlined in Scheme 1 provides an excellent route for the production of this desirable component of Georgywood.

We turn next to the enantioselective synthesis of the chiral enone **2** and its antipode *ent*-**2**. This synthetic problem was considerably more challenging than the synthesis of Georgywood, and a number of approaches that seemed feasible failed. It should be mentioned that there is no published synthesis of **2** or *ent*-**2**.[5b,12] An effective enantioselective synthesis of **2** is summarized in Scheme 2. The key enantioselective step again was the (*S*)-oxazaborolidinium cation (**5**)-catalyzed Diels−Alder reaction, in this instance using 1,3-butadiene and (*E*)-2-methyl-2-butenal as components.[9] The required product, aldehyde **8**, was formed with 16:1 enantioselectivity and in 84% yield. Oxidation of **8** to the corresponding carboxylic acid ($H_2Cr_2O_7$, acetone-H_2O) and an iodolactonization−β-elimination sequence provided in good yield the unsaturated γ-lactone **9**, which was further converted to the methyl ester-enone **10** by sequential methanolysis and oxidation with pyridinium chlorochromate (PCC) in CH_2Cl_2 at 23 °C. Reaction of **10** with the cyanocuprate reagent prepared from 5-chloro-5-methyl-1-hexene[13] (**11**), lithium 4,4′-di-*tert*-butylbiphenylide, and cuprous cyanide in THF at −78 °C in the presence of Me_3SiCl[14] afforded diastereoselectively a conjugate adduct which, upon aqueous workup and simultaneous silyl ether cleavage, resulted in a single unsaturated keto ester in 76% yield. Ozonolysis of this product gave the required keto aldehyde **12** (91%). Acid-catalyzed aldol cyclization of **12** led to the bicyclic α,β-enone **13**. This enone was converted to the olefinic ester **14** (68% overall) by the sequence (1) *p*-toluenesulfonylhydrazone formation and (2) reduction−

(6) We are indebted to Dr. G. Fráter of Givaudan Dübendorf AG for a gift of racemic Georgywood.
(7) Fráter, G.; Bajgrowicz, J. A.; Kraft, P. *Tetrahedron* **1998**, *54*, 7633−7703.
(8) Nussbaumer, C.; Fráter, G.; Kraft, P. *Helv. Chim. Acta* **1999**, *82*, 1016−1024.
(9) (a) Corey, E. J.; Shibata, T.; Lee, T. W. *J. Am. Chem. Soc.* **2002**, *124*, 3808−3809. (b) Ryu, D. H.; Lee, T. W.; Corey, E. J. *J. Am. Chem. Soc.* **2002**, *124*, 9992−9993. (c) Ryu, D. H.; Corey, E. J. *J. Am. Chem. Soc.* **2003**, *125*, 6388−6390. (d) Zhou, G.; Hu, Q.-Y.; Corey, E. J. *Org. Lett.* **2003**, *5*, 3979−3982. (e) Ryu, D. H.; Zhou, G.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 4800−4802. (f) Hu, Q.-Y.; Rege, P. D.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 5984−5986. (g) Hu, Q.-Y.; Zhou, G.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 13708−13713.
(10) Although synthetic **1** was contaminated by about 10% of the (1*S*,2*S*)-diastereomer, there is no contribution of this impurity to odor since it is essentially odorless, as shown in a later section of this paper.
(11) Odor testing was carried out with several members of our research group, including the authors, with good agreement on the consensus evaluation of odor.
(12) See: Fráter, G.; Müller, U.; Schröder, F. *Tetrahedron: Asymmetry* **2004**, *15*, 3967−3972.
(13) Dragoli, D. R.; Burdett, M. T.; Ellman, J. A. *J. Am. Chem. Soc.* **2001**, *123*, 10127−10128.
(14) Corey, E. J.; Boaz, N. W. *Tetrahedron Lett.* **1985**, *26*, 6019−6022.

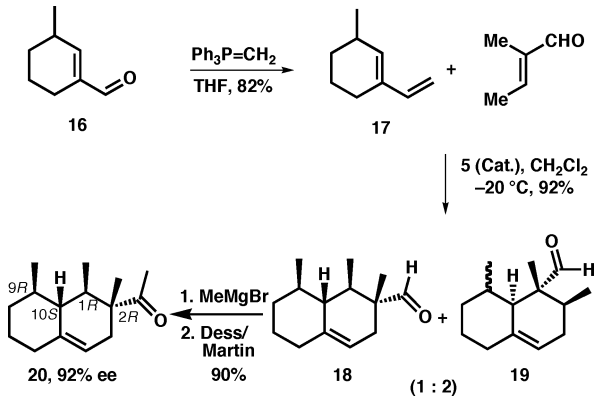
Scheme 2



elimination to form a diazene derivative which underwent electrocyclic fragmentation to give **14**.[15] Dibal-H reduction of **14** generated the corresponding primary alcohol which, upon oxidation with PCC, yielded the unsaturated aldehyde **15** (82% overall). Reaction of **15** with excess MeMgBr in THF and Dess−Martin periodinane oxidation in CH_2Cl_2 at 23 °C produced the dextrorotatory methyl ketone (+)-**2** (88% overall).[16] By a sequence paralleling that shown in Scheme 2 for the synthesis of (+)-**2**, but employing *ent*-**5** as catalyst in the initial Diels−Alder step, the levorotatory methyl ketone (−)-**2** was synthesized. The dextro ketone (+)-**2** was found to possess an intense woody odor that was clean and very pleasant.[11] In contrast, (−)-**2** exhibited only a very faint odor. Comparative tests in our laboratory indicated that the odor threshold for (+)-**2** is 20−30 times lower than that for (−)-**1**, the active enantiomer in racemic Georgywood. The human odor threshold value was reported by the Givaudan group as 5×10^{-12} g/L for (±)-**2** and 30×10^{-12} g/L for (±)-**1** (Georgywood).[5b] As a result of this work, the surprising fact emerges that the active enantiomer of Georgywood, (−)-**1**, and that of the powerful ingredient of Iso E Super, (+)-**2**, differ in configuration at C(2) (the ring carbon bearing the acetyl group). This observation has important implications with regard to olfactory perception that are discussed below. Because (+)-**2** has such a strong and clean woody odor, this enantiomer merits a name, and so we shall use the term "arborone" for this fragrant compound (from the Latin *arbor*, meaning wood or tree). Similarly, in the discussion below we shall refer to the odiferous enantiomer **1** as georgyone. In summary, arborone is the valuable component of Iso E Super, which is an ingredient in the commercial fragrances Fahrenheit, Trésor, Feminite du Bois, Declaration, Grojsman Accord, Narcisse, Bill Blass, and Dolce Vita.

(15) Kabalka, G. W.; Yang, D. T. C.; Baker, J. D. *J. Org. Chem.* **1976**, *41*, 574−575.
(16) Spectral data for (+)-(**2**) were in agreement with those reported for the racemate: Fráter, G.; Kraft, P. *Helv. Chim. Acta* **1999**, *82*, 1016.

Scheme 3



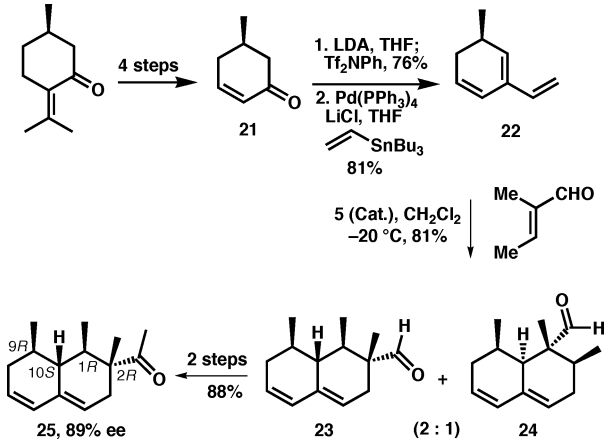
When **1** is treated with excess liquid ammonia (solvent) and anhydrous calcium sulfate at 30 °C for 24 h, it is converted cleanly to the corresponding imine. The odor of this imine is approximately the same as that of georgyone (**1**), and it is also pleasantly woody (and somewhat more persistent). The imine does not exhibit ammonia, amine, or *N*-heteroaromatic-type odor notes. These findings are consistent with the possibility that the acetyl oxygens of **1** and **2** (or the nitrogen of the imine) serve as H-bond acceptors when bound to the complementary OR.

We next turned our attention to the question of the role of the two geminal methyl groups at C(9) in arborone (**2**), specifically regarding the role of that methyl group that is cis to acetyl in **2**. The synthesis of the required molecule (**20**) is outlined in Scheme 3. Aldehyde **16** (racemic)[17] was converted by Wittig methylenation to the conjugated diene **17** which, when allowed to react with (*E*)-2-methyl-2-butenal in the presence of the chiral catalyst **5** at −20 °C for 24 h, led to a mixture of Diels−Alder adducts **18** and **19** (ratio 1:2) in 92% total yield.[18] These positional isomers were difficult to separate. However, after reaction of the mixture with methylmagnesium bromide, the corresponding methyl carbinols were produced which could be readily separated by chromatography on silica gel. Dess−Martin oxidation of each carbinol gave the corresponding methyl ketones. The desired desmethyl arborone **20** was thus obtained in 90% yield and 92% ee. Enone **20** possesses an intense warm woody-amber odor in common with **1** and **2**. Its odor also has a slight fresh minty note. In contrast, the enantiomer of **20**, which was synthesized by the process outlined in Scheme 3 using *ent*-**5** as catalyst in the Diels−Alder step, possesses a relatively weak odor. The 6,7-didehydro analogue of **20**, enone **25**, was synthesized using a similar strategy, as shown in Scheme 4.[19] The odor of **25** was quite similar to that of **20**, indicating that the additional $\Delta^{6,7}$-double bond makes very little difference.

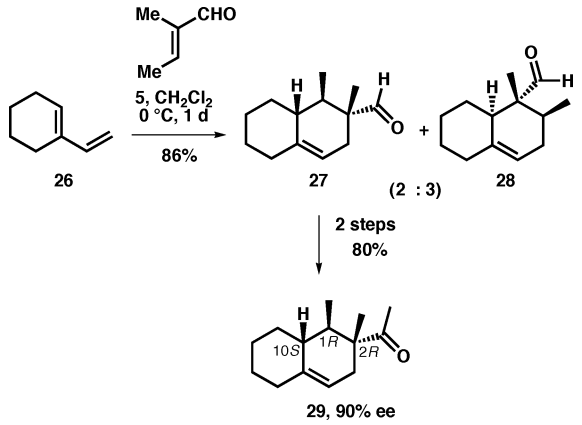
Comment is required on the formation of the two positional isomers **18** and **19** in the Diels−Alder reaction leading to **20**

(17) The aldehyde **16** was synthesized by the sequence (1) C(6)-formylation of 2-methylcyclohexanone with methyl formate and NaH in benzene, (2) conversion to the ethoxyethyl ether with ethyl vinyl ether−H_3PO_4, and (3) reduction with $NaBH_4$ in EtOH followed by H_2SO_4−THF−H_2O. For precedent, see: Kavanobe, T.; Kogami, K.; Hayashi, K.; Matsui, M. *Agric. Biol. Chem.* **1984**, *48*, 461−464.
(18) The Diels−Alder reaction can be carried out using an excess of the racemic diene **17** because of a faster rate of reaction for the (*R*)- vs the (*S*)-enantiomer, an interesting illustration of the efficacy of catalyst **5**. This is an unusual example of kinetic resolution in a catalytic enantioselective Diels−Alder reaction.
(19) For the synthetic procedure for pulegone → enone **21**, see: Caine, D.; Procter, K.; Casall, R. A. *J. Org. Chem.* **1984**, *49*, 2647−2648.

Scheme 4



Scheme 5

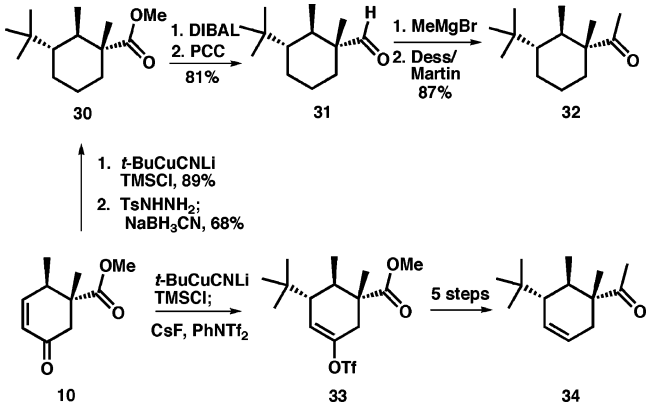


(Scheme 3). From the mechanistic model for this reaction with catalyst **5**, it is clear that regioisomer **18** is disfavored by a steric repulsion in the transition state between the methyl substituent on the diene and the β-methyl group of the dienophile. As a result, the regioisomeric adduct **19** predominates 2-to-1 over **18**. This steric effect is related to the repulsion between the methyls at C(1) and C(9) in the adduct **20**.

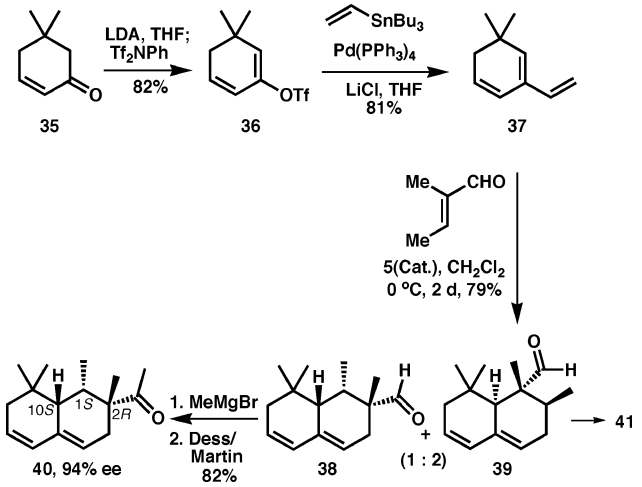
Scheme 5 outlines the synthesis of the analogue of arborone lacking both methyl groups at C(9) (i.e., in the *gem*-dimethyl subunit) of **2**, enone **29**. An enantioselective synthesis proceeded from 1-vinylcyclohexene and (*E*)-2-methyl-2-butenal as Diels–Alder components with catalyst **5**, which gave adduct **27** along with the position isomer **28**. Addition of methylmagnesium bromide to the mixture, chromatographic separation, and oxidation gave enone **29**. This analogue of **2**, lacking both methyls of the *gem*-dimethyl unit, possesses very little odor. Thus, replacing the (9*R*)-methyl subunit of **20** with hydrogen as in **29** essentially abolishes odor, a surprising finding.

In view of the critical role of one of the geminal dimethyl groups at C(9) of arborone, as deduced from the great difference observed in the odors of **2**, **20**, and **29**, we investigated the effect of allowing the critical methyl group to move relative to the cyclohexene ring. This was done by synthesizing the monocyclic enones **32** and **34** from the keto ester **10**, as shown in Scheme 6. The starting material **10** had already been synthesized as an intermediate for the synthesis of (+)-**2** (Scheme 2). Conjugate addition of *tert*-butyl to **10** afforded stereoselectively a single keto ester, which was converted to ester **30** by the sequence (1) tosylhydrazone formation and (2) reduction by sodium cyanoborohydride in dimethylformamide. Reduction of **31** with excess diisobutylaluminum hydride in toluene and oxidation with pyridinium chlorochromate (PCC) in CH_2Cl_2 afforded the aldehyde **31**, which was further converted to the methyl ketone **32** by the usual methylation–oxidation sequence. Reaction of *t*-BuCuCNLi and Me_3SiCl at −78 °C gave the TMS enol ether of the conjugate addition product which, when treated with CsF and $PhNTf_2$,[20] produced the vinyl triflate **33**.[20] The triflate was replaced by hydrogen (Bu_3SuH, $Pd(PPh_3)_4$, THF), and the resulting ester was converted to **34** by a sequence paralleling **30** → **32**. Neither **32** nor **34** possessed the strong woody odor of (−)-**1** or (+)-**2**, but instead they had a weak odor reminiscent of methyl ketones such as farnesylacetone. From this result it is clear that the bicyclic structure of **1** or **2** and a fixed spatial location of the critical methyl groups are important for odor.

Scheme 6



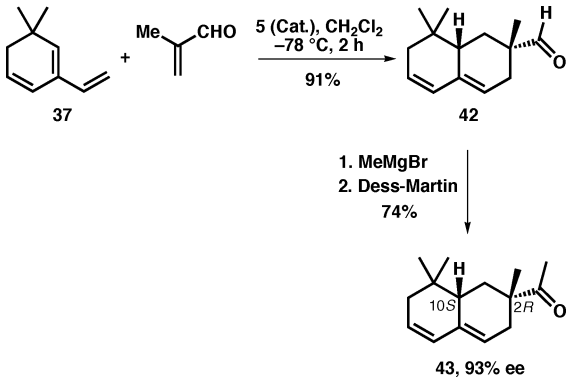
Scheme 7



We have also determined that the spatial orientation of the methyl group attached to C(1) of (+)-**2** is important for odor by examining the synthetic analogue **40**, the synthesis of which is summarized in Scheme 7. Enone **35** was converted via the vinyl triflate **36** to the triene **37**. Diels–Alder reaction of **37** with (*E*)-2-methyl-2-butenal using the chiral catalyst **5** afforded the required adduct **38**, along with the position isomeric adduct **39**. Methylation of aldehyde **38** with MeMgBr and periodinane oxidation provided the enone **40**. The structure and absolute configuration of **40** were established by conversion to the

(20) Mi, Y.; Schreiber, J. J.; Corey, E. J. *J. Am. Chem. Soc.* **2002**, *124*, 11290–11291.

Scheme 8



crystalline *p*-toluenesulfonylhydrazone derivative and X-ray diffraction analysis.[21,22] The absolute configuration of **38** is that predicted by the mechanistic model for catalyst **5** and an *endo*-formyl pathway.[9] In contrast to arborone (**2**) or the dienone **25** (see Scheme 4), the (1*S*)-methyl analogue **40** possesses at best only a very faint odor. Thus, we conclude that the (*R*)-configuration at C(1) of arborone (**2**) or georgyone (**1**) is critical to their binding at the corresponding woody-type OR.

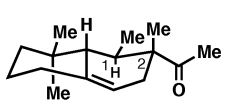
The formation of adduct **38** from diene **37** and (*E*)-2-methyl-2-butenal, which was not expected, can readily be explained by the intervention of Lewis-acid-catalyzed $E \rightarrow Z$ isomerization of the enal dienophile prior to the Diels−Alder reaction. This explanation was verified by a control experiment in which (*E*)-2-methyl-2-butenal was treated with catalyst **5** at 0 °C in CD_2Cl_2 (conditions for the conversion of **37** to **38** and **39**). Greater than 95% *E*-to-*Z* isomerization was observed within 30 min (by ^{1}H NMR analysis).

The aldehyde **39** was transformed in two steps (addition of CH_3Li, followed by periodinane oxidation) to the corresponding methyl ketone (**41**), the odor of which was not woody but camphor-like.

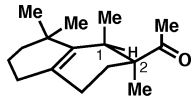
The importance of the (1*R*)-stereocenter in arborone (**2**), georgyone (**1**), and dienone **25** to the property of woody odor was further confirmed by the synthesis of methyl ketone **43**, which lacks the C(1)-methyl substituent of arborone or analogue **25**. The pathway of synthesis is outlined in Scheme 8. As expected, **43** does not exhibit a woody odor.

Discussion

The syntheses of arborone (**2**, Scheme 2) and of the structurally related odorants georgyone (**1**, Scheme 1), **20** (Scheme 3), **25** (Scheme 4), **29** (Scheme 5), **32**, **34** (Scheme 6), and **40** (Scheme 7) provide an abundance of information connecting the chemical structure of these conformationally well-defined molecules with odor, as perceived by humans. From the odor evaluation of these compounds and their enantiomers, it is clear that most of the structural units of arborone (**2**), the most potent odorant, are essential for the clean woody odor. These essential features of **2** include the absolute configuration, the flat, bicyclic ring system, the equatorial methyls at C(1) and C(9), and the acetyl group at C(2). In contrast, the axial methyl group at C(9) of **2** is not critical. The conformational rigidity of **2** and the fact that its odor can be detected in the picomolar range[5b] indicate that it binds very tightly to one or more complementary OR sites. It is significant that georgyone (**1**) has a similar (but somewhat less powerful) odor even though the configuration at C(2) and the spatial orientation of acetyl are opposite for **1** and **2**. We believe that these facts have important implications for the structural features of the receptor binding sites of **1** and **2**. These will be analyzed in a following section, but first it is necessary to consider the present state of knowledge regarding the mechanisms by which the occupancy of ORs by odorants is signaled to the brain as useful information.



2



1

As indicated in the introductory section, the wiring diagram for odor sensing and signaling is remarkably simple, as has been shown by studies in mice. Each receptor type is connected via the corresponding neuron to a specific glomerulus for that particular OR/ON type, and all the ONs converge on the dedicated glomerulus. The signal to the glomerulus is generated by ligand-induced conformational change in the OR, activation of adenyl cyclase by the associated G-protein leading to the formation of cyclic AMP and cyclic AMP gating of ion channels. These ion movements into the ONs generate an action potential and electrical pulses (spikes) that travel axonally to the glomeruli. Although there is some evidence for the formation of second messengers other than cyclic AMP (e.g., IP_3 and cyclic GMP), it now appears that cyclic AMP-mediated signaling strongly dominates.[23,24] Thus, it is clear that if several ligands were to bind to the same receptor type, they would produce one type of signal, although the total signal amplitude would obviously depend on the total number of occupied and activated receptors. Given that each receptor produces only one type of signal regardless of ligand, the diversity of signaling to allow for distinguishing between thousands of odorants must be due to ligand binding to multiple receptors and a resultant overall signal that is diversified by the many possible combinations of receptors, as has been proposed previously.[1b,2d,25] For an odorant that binds to and activates 5 of the 340 human ORs, the number of possible combinations (n) and different signals would be very large (over 30 billion), specifically:

$$n = \frac{340!}{(5!)(335!)} = 36.8 \times 10^9$$

Most of the data for ligands that interact with multiple receptors have been obtained with simple, conformationally mobile compounds, such as acyclic carboxylic acids, alcohols, and aldehydes.[2d,25] It was not clear whether conformationally

(21) The results that are summarized in Scheme 7 also show that this particular Diels−Alder reaction is not useful for the synthesis of (+)-**2**, although we entertained this possibility when considering alternative synthetic routes to **2** that are simpler than the one set out in Scheme 2.

(22) For details of X-ray diffraction analysis of the tosylhydrazone of **38**, see the Supporting Information.

(23) (a) Takeuchi, H.; Kurahashi, T. *J. Gen. Physiol.* **2003**, *122*, 557−567. (b) Barry, P. H. *J. Gen. Physiol.* **2003**, *122*, 247−250. (c) Madrid, R.; Delgado, R.; Bacigalupo, J. *J. Neurophysiol.* **2005**, *94*, 1781−1788.

(24) For a summary of older work on the possibility of multiple second messengers, see: Schild, D.; Restrepo, D. *Physiol. Rev.* **1998**, *78*, 429−466.

(25) (a) Uchida, N.; Takahashi, Y. K.; Tanifuji, M.; Mori, K. *Nature Neurosci.* **2000**, *3*, 1035−1043. (b) Takahasi, Y. K.; Kurosaki, M.; Hirono, S.; Mori, K. *J. Neurophysiol.* **2004**, *92*, 2413−2427. (c) Araneda, R. C.; Kini, A. D.; Firestein, S. *Nature Neurosci.* **2000**, *3*, 1248−1255.

rigid odorants such as arborone (**2**) and georgyone (**1**), which are quite nonpolar and lipophilic, could activate multiple receptors at the very low concentrations that produce an odor signal. In collaboration with Prof. Markus Meister and his group (Harvard University), we have begun an investigation of this question using live mice (which have ca. 1000 ORs).[26] This work involves the optical detection of olfactory glomerulus activation by fluorescence microscopy.[27] Although this research is ongoing and still incomplete, compelling evidence has been obtained that **1** and **2** each bind strongly to *at least 10* different types of ORs. About half of these receptors are common to **1** and **2**, and the rest are specific for either **1** or **2**. *ent*-Georgyone, on the other hand, showed binding to *only one* observable receptor which was different from those activated by **1** or **2**.[28] The receptor activated by *ent*-georgyone is therefore probably not one that sends a wood odor signal. Our studies with the synthetic, enantiomerically pure products of this research have provided clear evidence that individual ORs possess multiple functional binding sites and, further, that one OR can bind a range of *different* molecules in these sites even at low ligand concentration (see also ref 2d).

The ORs are G-protein-coupled receptors having seven transmembrane (7TM) α-helical domains. The sequences of the human and mice ORs place them in the A class of G-protein-coupled receptors, the best known member of which is rhodopsin, the receptor involved in visual sensing and signal transduction.[29]

At this stage of our research, the specific human or mouse ORs that are activated by **1** and **2** are not known. Although the glomeruli of mice that are activated by **1** and **2** have been identified, the identity of the ORs to which they are connected (and by which they are activated) is currently unknown. Thus, even though the amino acid sequences are known for the various human and mouse ORs, it is not clear how to determine experimentally the ORs or binding sites for **1** and **2**. Nonetheless, we have developed a reasonable working hypothesis for the mode of binding of **1** and **2** (and active woody odorant analogues such as **20** and **25**) with their complementary ORs based on the stringent structural requirements for woody odor that have been demonstrated in this study. In particular, from the structural requirements for odor summarized just above, it is clear that there is a very precise fit between the region of the rigid bicycles **1** and **2** that contain the methyl substituents and one of the helical TM domains. From extensive modeling studies which examined possible lipophilic cavities (based on the a-x-x-b binding motif) on an α-helix, we have deduced that by far the most likely interaction for strong and specific hydrophilic interaction with the methylated region of **1** and **2** would occur for an α-helical section having 1,4-located leucine (L) or isoleucine (I) residues, e.g. L-X-Y-L or L-X-Y-I. The L-L or L-I side chains form a pocket which is nicely complementary

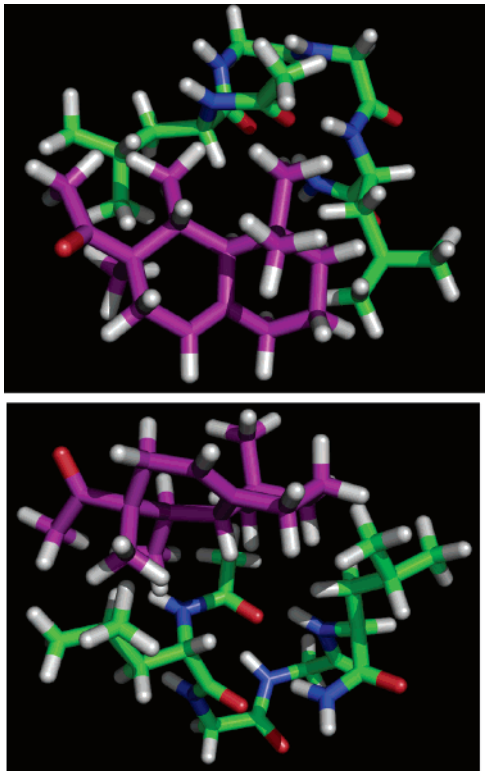


Figure 1. Top and side views of arborone (**2**) docked on an α-helical L-G-G-L motif.

to the methyl-bearing regions of **1** and **2** (e.g., C(1), C(2), C(10), C(9), and C(8), i.e., the top part of formula **2**). Analogues **29**, **32**, **34**, **40**, and **43** would not be expected to bind as well in this pocket, in agreement with their lack of the strong woody odor characteristic of **1** and **2**. We also make the reasonable prediction that the acetyl group of **1** and **2** binds to the 7TM receptor by serving as an H-bond receptor which accepts a proton from a carboxamide (from N or Q), a hydroxyl (S or T), or a carboxylic (D or E) side chain in a different α-helix. Our studies show that the acetyl H-acceptor group is critical for woody odor since its substitution by CHO, $CH(OH)CH_3$, or $COOCH_3$ results in essentially odorless compounds, whereas replacement by $CH_3C{=}NH$ (i.e., georgyone imine) preserves odor. Thus, we picture **1** or **2** as first binding to the receptor at a TM helix having the L-X-Y-L subunit and that complex recruiting a second TM helix having the proton donor function and also a hydrophobic site for complementary binding to the C(3)−C(8) sections of **1** and **2** (see Figure 1). It is possible that a third TM α-helix may contribute to this hydrophobic binding pocket. This model easily explains why **1** and **2** have different spatial orientations of the acetyl substituent, since the second α-helix that provides the donor H can be juxtaposed with the first α-helix in a somewhat different alignment (and even use a different residue as the H-donor group). Some evidence in support of this postulate has been provided by data reported recently by Katada and Touhara et al.[30] These workers studied a mouse OR that was known to bind strongly to the odorant eugenol (2-methoxy-4-allylphenol). From homology modeling based on the rhodopsin structure[29] and extensive site-directed mutagenesis on the mouse eugenol receptor, together

(26) This collaborative research by the authors, Prof. Meister and Mr. Edward Soucy, will be described in detail elsewhere.
(27) Bozza, T.; McGann, J. P.; Mombaerts, P.; Wachowiak, M. *Neuron* **2004**, *42*, 9−21.
(28) Only about 20% of the mouse olfactory glomeruli are accessible for study using the optical analysis technique. It is thus possible that additional glomeruli are activated by olfactory ligands **1** and **2**.
(29) (a) Fredriksson, R.; Lagerström, M. C.; Lundin, L.-G.; Schiöth, H. B. *Mol. Pharmacol.* **2003**, *63*, 1256−1272. (b) Ballesteros, J. A.; Shi, L.; Javitch, J. A. *Mol. Pharmacol*. **2001**, *60*, 1−19. (c) Ballesteros, J. A.; Palczewski, K. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 561−574. (d) Mirzadegan, T.; Benkö, G.; Filipek, S.; Palczewski, K. *Biochemistry* **2003**, *42*, 2759−2767.
(30) Katada, S.; Hirokawa, T.; Oka, Y.; Suwa, M.; Touhara, K. *J. Neurosci.* **2005**, *25*, 1806−1815.

with measurements of ligand binding to the various mutants of the eugenol, they concluded that TM3, TM5, and TM6 are involved in forming the binding pocket. They showed that N207, F206, and L212 of TM5, S113 of TM3, and L259, I256, and F252 of TM6 are critical for eugenol binding and receptor activation.[30] The TM6 interaction agrees with our model (note the I-X-Y-L subunit of TM6).[30,31]

This research supports the view that even conformationally rigid, strongly binding small odorant molecules bind to a surprisingly large number of olfactory receptors and that odor discrimination is fundamentally a combinatorial phenomenon. Although it may seem surprising that any individual OR can be activated by a number of ligands, it is not unreasonable that ORs which can accommodate multiple ligands would be evolutionarily favored. In this respect, such sensory ORs conform to different requirements than regulatory receptors (e.g., insulin, glucocorticoid, serotonin, prostaglandin, etc.) which need to respond to only one particular molecule. By this way of thinking, certain cellular receptors that can accommodate a range of ligands, such as peroxisome proliferator-activated receptors (PPARs), may be regarded as sensory receptors that sample multiple compounds in the biochemical environment and respond accordingly.[32] Such receptors, including ORs, are probably sufficiently versatile and spatially mobile to provide a variety of binding sites for ligands by either conformational induction or selection by the ligand.[33] The possibilities that spring from the 7TM α-helical assembly of G-protein-coupled receptors because of alternative ways of clustering the α-helixes about a set of different ligands provide a versatility that is ideal for a "sensing" receptor. The existence of multiple binding sites for multiple related ligands clearly does not preclude a range of ligand binding affinities or very strong binding for a ligand that provides an ideal fit for a particular binding pocket. Our analysis of alternative modes of binding of **1** and **2** to ORs has led to the surmise that one of the TM α-helixes of the OR may serve as the initial binding contact and that a second and third α-helix may be recruited subsequently to form the ligand-occupied activated receptor complex.

Although our biological studies are still at an early stage, it is of some interest that glomeruli that are activated by woody odorants seem to lie together as close neighbors on the olfactory bulb of the mouse, even if they are chemically and structurally different; for example, longifolene (which has a woody odor) activates some of the same glomeruli and also neighboring glomeruli as **1** and **2**. This result suggests the possibility that similar odorants may activate neighboring glomeruli, an intriguing aspect of olfactory organization of information. The chemical studies described herein provide a tool for probing not only receptor binding but also the spatial organization of olfactory glomeruli, a new frontier of neuroscience.

Acknowledgment. We are grateful to Professor Markus Meister, Dr. Edward Soucy, and Mr. Florin Albeanu for the mouse olfactory data and to Mr. Kevin Phillips and Mr. Brian Tse for assistance with the graphics.

Supporting Information Available: Procedures for the reactions shown in Schemes 1−8 and characterization data (PDF); X-ray crystallographic data for the tosylhydrazone of **38** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(31) For other interesting recent studies on ligand binding to ORs with simple compounds (e.g. 1-hexanol), see: (a) Floriano, W. B.; Vaidehi, N.; Goddard, W. A., III; Singer, M. S.; Shepherd, G. M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10712−10716. (b) Frimurer, T. M.; Ulven, T.; Elling, C. E.; Gerlach, L.-O.; Kostenis, E.; Högberg, T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3707−3712. (c) Man, O.; Gilad, Y.; Lancet, D. *Protein Sci.* **2004**, *13*, 240−254.
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