dom. While transplants are innervated by the host's visual afferents only if they lie sufficiently close to them, they are never totally innervated from the eye or visual cortex even if embedded in the tissue these afferents normally supply. As such they exhibit a form of specificity. It is tempting to suggest that those areas of the transplant that receive visual inputs correspond to the visual layers of normal animals and that specific affinities which some cells appear to demonstrate for visual afferents in intact tissue are preserved in the transplantation procedure. Conversely, those areas lacking innervation correspond to cell layers that would not normally be innervated by visual afferents.

The question arises whether the special affinities suggested by this work are still expressed if tissues are taken at progressively earlier ages or if they can be abolished by various manipulations in vitro before transplantation. In addition, it is not known whether tissues such as visual or somatosensory cortex also display any affinity for optic axons if placed close to their area of distribution. These questions are approachable with the present system since further studies have indicated that younger tectal tissue and tissue from various cortical areas exhibit interconnections with the host colliculus (*12*), and that such interconnections occur even if cortical or tectal tissue is maintained in vitro prior to transplantation. Ultimately, it should be possible to assess the extent to which specific connections between brain regions reflect special affinities between certain cell types (for instance, "visual" cells as opposed to "somatosensory" cells) and whether such affinities may be limited in their expression by the particular growth patterns of the fiber tracts interconnecting them.

RAYMOND D. LUND
Departments of Biological Structure and Neurological Surgery, University of Washington, Seattle 98195
STEPHEN D. HAUSCHKA
Departments of Biochemistry and Zoology, University of Washington

References and Notes

1. R. W. Sperry, *J. Neurophysiol.* **7**, 57 (1944).
2. M. I. Olson, and R. P. Bunge, *Brain Res.* **59**, 19 (1973); S. M. Crain and E. R. Peterson, *Science* **188**, 275 (1975).
3. R. W. Sperry, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 703 (1963).
4. A. J. Barbera, R. B. Marchase, S. Roth, *ibid.* **70**, 2482 (1973); D. Gottlieb, R. Merrell, L. Glaser, *ibid.* **71**, 1800 (1974); A. A. Moscona, in *The Cell Surface in Development*, A. A. Moscona, Ed. (Wiley, New York, 1974), pp. 67–99; S. H. Appel, *Exp. Neurol.* **48**, 52 (1975); U. Rutishauser, J.-P. Thiery, R. Brackenbury, B.-A. Sela, G. M. Edelman, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 577 (1976).
5. R. D. Lund and J. S. Lund, *Science* **171**, 804 (1974); L. S. Jen and R. D. Lund, unpublished observations.
6. R. M. Gaze, *Br. Med. Bull.* **30**, 116 (1974); R. K. Hunt and M. Jacobsen, *Curr. Top. Dev. Biol.* **8**, 203 (1974); G. Lynch, S. Deadwyler, C. Cotman, *Science* **180**, 1364 (1973); G. E. Schneider and S. R. Jhaveri, in *Plasticity and Recovery of Function in the Central Nervous System*, D. G. Stein, J. J. Rosen, N. Butters, Eds. (Academic Press, New York, 1974), pp. 65–109.
7. Transplants into fetal animals can be rejected, there apparently being an interaction with maternal antibodies that cross the placental barrier.
8. G. D. Das, *TIT J. Life Sci.* **4**, 93 (1974); *Adv. Neurol.* **12**, 181 (1975).
9. R. D. Lund, *Invest. Ophthalmol.* **11**, 434 (1972).
10. _____ and A. H. Bunt, *J. Comp. Neurol.* **165**, 247 (1976).
11. R. P. Fink and L. Heimer, *Brain Res.* **4**, 369 (1967); R. D. Lund and L. E. Westrum, *Science* **151**, 1397 (1966); W. M. Cowan, D. J. Gottlieb, A. E. Hendrickson, J. L. Price, T. A. Woolsey, *Brain Res.* **37**, 21 (1972).
12. Connections are shown with a neurofibrillar stain, but their source has not yet been identified.
13. We thank R. P. Wise and E. LaBossiere for valuable technical assistance and J. S. Lund for critical review of the manuscript. Supported by PHS grant EY-00596 to R.D.L. and by a research career development award to S.D.H.

1 April 1976

Potential New Artificial Sweetener from Study of Structure-Taste Relationships

Abstract. *4-(Methoxymethyl)-1,4-cyclohexadiene-1-carboxaldehyde* syn-*oxime is a new sweetening agent developed by systematic synthesis and taste evaluation of 80 new oximes analogous to the little-used oxime sweetener, perillartine.*

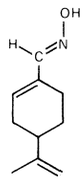
Sweet tasting substances display such a diversity of chemical structure that it has been difficult to relate sweetness with structure in any unified theory, or design a new sweetener on a rational basis. Consequently, most sweetening agents were discovered by accident. All have imperfect properties as sugar substitutes, and new sweeteners are in demand. Aldoxime **44** (Fig. 1) is a new noncaloric sweetener that has 450 times (on the basis of weight) the sweetening power of sucrose, lacks the bitterness of saccharin, and has potential applicability for all sweetener uses (*1*). It was designed as an analog of perillartine **1** (*2, 3*), which has been used only as a sweetener for tobacco because of its very low water solubility, appreciable bitterness, and menthol-licorice off-tastes.

Perillartine was a useful starting point for a systematic study of taste-structure relationships (*4*). We assumed that the aldoxime moiety in **1** was the functional group responsible for sweetness. Then the rest of the molecule could be designated a "carrier" group, where changes in structure might be made. Perillartine has a high calculated potency of taste (*5, 6*), but because of its low solubility in water only a weak taste is actually attainable in solution. In designing and synthesizing new analogs (Fig. 1) our objective was to maintain high potency, increase the solubility in water, and minimize or eliminate nonsweet tastes (*7, 8*).

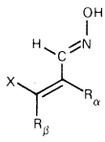
Both the aldoxime moiety and the α, β-olefinic unsaturation [as in **2**, where X = H, but $R_\alpha \neq H$ (*4, 9*)] were found to be essential for sweetness. Rather drastic changes were permitted in the carrier group, such as removal of the side chain (**26**), change of ring size (**17**), or even cleavage of the ring (**4**) (*10*). Unpleasant phenolic medicinal off-tastes developed in **26**, **17**, and **4**, but appreciable sweetness and taste potency were retained. Complete loss of sweetness in **40** and **41** (ketoxime analogs of **4** and **26**) confirmed that an aldoxime is required. Need for the free oxime OH group was confirmed by the complete insolubility and tastelessness of the *O*-methyl oxime **5** of perillartine.

Fig. 1 (right). Chemical structures of oximes and their properties. Numbers under each structure are oxime solubilities (in molarity, *M*), taste potencies indicated by × (times sucrose), and ratios of the percentage of sweetness to that of bitterness estimated from the taste qualities (total 100 percent) observed for each compound. Taste screening data were single responses from four to six experienced panelists, on oxime solutions at one or two concentrations producing observed taste intensities from 0.2 to 2.0 (usually 0.5 to 1.5) relative to 0.25*M* sucrose as 1. On compounds of further interest, up to ten replications at three to four concentrations were obtained. Maximum water solubilities are given in molarity at 25°C. The potency times sucrose on a mole/mole basis = [observed intensity of oxime solution/molarity of oxime] ÷ [observed intensity of sucrose reference solution/molarity of sucrose]. The ratios listed = (percent of taste identified as sweet)/(percent identified as bitter). Other tastes = 100 percent − (sweet + bitter). There was no sourness (except for **6**) or saltiness; but menthol, anise, licorice, coconut, and mint qualities were common. Fruit-berry tastes were noted on **11** and **44**. Unpleasant off-tastes were variously described as chemical, oily, phenolic, medicinal, or peppery on **3**, **4**, **8**, **9**, **13**, **17**, **18**, **25**, **26**, **27**, **34**, **38**, **39**, **46**, **47**, **49**, and **51**. Although not predominantly sweet, **3** and **27** are listed with sweet compounds for comparison. One-time responses on sodium saccharin and calcium cyclamate gave potencies of 185 and 30, and ratios of percent sweet to the percent bitter were 93/7 and 80/0.

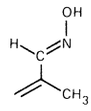
SWEET



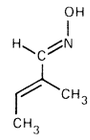
1
0.0005M
370x
60/25



2

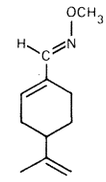


3
0.60M
8x
15/18

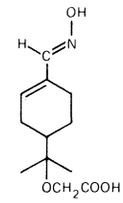


4
0.15M
40x
55/8

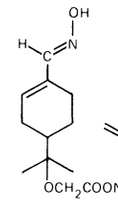
NONSWEET



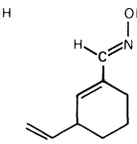
5
insol



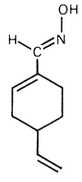
6
0.002M
95x
17/12 (sour)



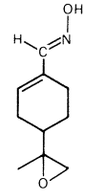
7
0.005M
10x
0/38



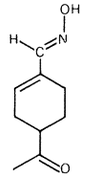
8
0.0002M
175x
14/34



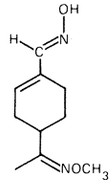
9
0.0002M
1150x
50/10



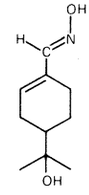
10
0.003M
50x
65/10



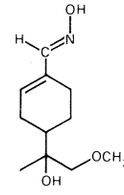
11
0.011M
12x
23/14



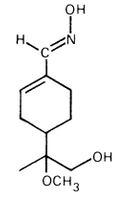
12
0.002M
80x
55/20



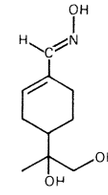
13
0.027M
8x
4/18



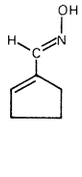
14
0.012M
4x
6/70



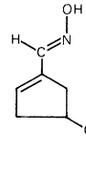
15
0.016M
11x
4/93



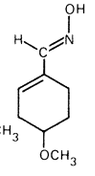
16
2.0M
30x
5/73



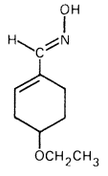
17
0.080M
55x
48/7



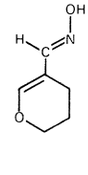
18
0.006M
24x
39/9



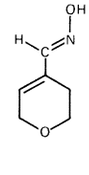
19
0.050M
8x
42/26



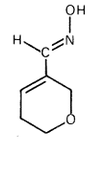
20
0.011M
16x
45/28



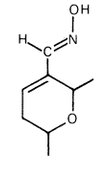
21
0.080M
3x
22/19



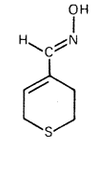
22
0.70M
1.5x
0/50



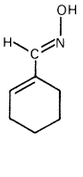
23
0.080M
2x
2/65



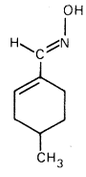
24
0.023M
10x
0/36



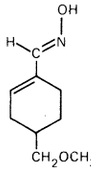
25
0.055M
20x
0/70



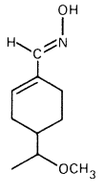
26
0.017M
55x
40/16



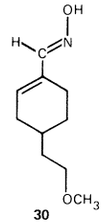
27
0.0006M
250x
16/20



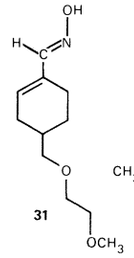
28
0.030M
52x
53/22



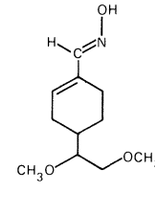
29
0.003M
80x
50/20



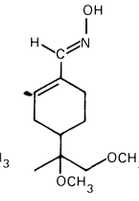
30
0.0005M
Tasteless



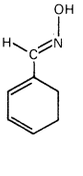
31
insol



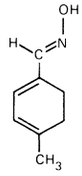
32
0.022M
8x
0/0 (menthol)



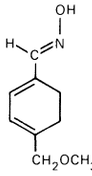
33
0.009M
50x
0/78



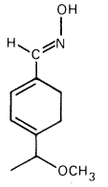
34
0.018M
90x
50/15



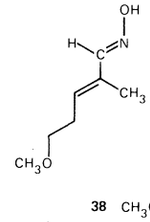
35
0.0006M
150x
22/13



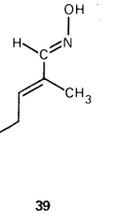
36
not
isolated



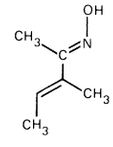
37
(0.004M)
135x
65/7



38
0.14M
4x
2/40



39
0.025M
17x
4/26



40
0.001M
140x
0/57

41
0.0004M
410x
0/66

42
0.015M
200x
70/3

43
0.0006M
500x
78/3

44
0.020M
225x
90/2

45
0.004M
300x
92/1

46
0.0004M
320x
34/40

47
0.006M
33x
12/16

48
0.003M
28x
2/52

49
(0.02M)
140x
0/75

50
(0.004M)
92x
0/67

51
0.012M
320x
0/50

Often there was an inverse relation between water solubility and taste potency, which had to be circumvented for a successful outcome. Compounds with low solubilities often had high potencies, but structure changes that improved solubility often reduced the potency, so that a strong taste in solution was still hard to attain. For example, alcoholic OH groups in the perillartine side chain (**13** to **16**) gave predictable increase in water solubility, but both sweetness and taste potency were lost. Therefore, we tried less strongly polar substituents to gain solubility. Results were more favorable with demethylperillartine **9**, the epoxide **10** (*11*), the ketone **11**, and its *O*-methyl ketoxime **12**. Solubility was not so strongly increased as with OH, but sweetness was retained. There was bitterness and the usual menthol-anise tastes, but not the phenolic-medicinal off-tastes of **26**, **17**, and **4**. Ketone **11** achieved good solubility without an alcoholic OH; its potency and sweetness were low, but there were pleasant fruit-berry tastes. These compounds **9** to **12** were not useful sweeteners (*12*), but taken together their properties suggested the further use of weakly polar groups in the carrier moiety. Use of ring oxygen in structures **21** to **24** seemed consistent with this idea, but although solubility was good, both sweetness and potency of taste were lost with these dihydropyrans (as well as the sulfur compound **25**).

Better direction was provided by the OCH_3 group in **19**. Importance of ether oxygen in the side chain was seen when we compared the 4-OCH_3 compound **19** with its 4-CH_3 analog **27**. There was little sweetness in the taste of **27** (16 percent sweet), but the taste quality of **19** was nearly half sweet (42 percent), and the disagreeable phenolic-medicinal off-tastes of **26** (a common point of departure for **19** and **27**) were absent. As usual, potency decreased as solubility increased (for **19**); and potency increased as solubility decreased (for **27**). The next higher homologs of **19** were the 4-ethoxy ether **20** and the isomeric 4-methoxymethyl ether **28**. Surprisingly, **28** was two to three times better than **20** in both solubility and taste potency, the first time these had increased together. Thus, in going from **19** to **28**, a carbon atom was inserted with only a 40 percent drop in solubility but a sixfold increase in potency. We concluded that ether oxygen was best incorporated as OCH_3. The next homolog was **29**, with a branched side chain; sweetness was unchanged, but the increase in potency was accompanied by a tenfold drop in solubility. Comparison of the two racemic forms of **29** showed the importance of spatial orientation of the side chain; one racemate had improved potency (110 ×) and sweetness (77 percent), with corresponding losses in the other. The importance of OCH_3 and its placement in the properties of **19**, **28**, and **29** (*13*) is consistent with the concept of a "third binding site" required in sweet molecules according to the Kier hypothesis (*14*). Misplacement of this site may explain the loss of sweetness in compounds **21** to **25**. Its absence may explain the off-tastes in **4**, **17**, and **26**. Perhaps for similar reasons the methoxymethyl side chain on the cyclopentene ring in **18** did not provide the anticipated improvement over **17**; sweetness and potency were less in **18** and there was an unexplained loss in solubility.

Extending the side chain beyond that in **29** resulted in sudden and complete loss of sweetness, even with **30**, an isomer of **29**, and regardless of the incorporation of one or two OCH_3's in **31**, **32**, and **33**. The adverse effect may be due to increased distance of the third binding site, and to increased flexibility and bulkiness of the side chain. Structural flexibility probably explains loss of sweetness in open-chain oximes homologous to **4**, even when OCH_3 is present as in **38** and **39**.

Conversely, increased structural rigidity may explain improved sweetness in cyclohexadiene analogs of compounds **26** to **29**. Ring rigidity increases from cyclohexene to 1,3-cyclohexadiene to 1,4-cyclohexadiene, and sweetness improved dramatically in the same order (*15*). In the series **26**, **34**, and **42**, and similarly with each side chain, the 1,4-cyclohexadiene was highest in sweetness and lowest in bitterness and off-tastes. Predictably, the 1,4-dienes **44** and **45** with OCH_3-bearing side chains were best, with almost no bitterness and a slight menthol-licorice quality accompanying sweetness. The 1,4-dienes also had the best taste potency. Solubility depended on the side chain and varied little with the nature of the ring. For its combination of good solubility allied with potency and quality of sweetness, **44** stood out as a potentially valuable sweetening agent (*16*). Sugar-sweetened beverages contain up to 12 percent sucrose. The same degree of sweetness is attained in a 0.024 percent solution of **44**. The potency of **44**, calculated in practical terms on a weight basis, is 450 times that of sucrose; the potency is 300 for saccharin and 30 for cyclamate.

Further evidence for the highly specific structural requirements for sweetness was provided by the loss of sweetness and even taste potency in **8** and **47** to **50** (positional isomers of **9**, **18**, **29**, **44**, and **45**, respectively). Increased rigidity with a bridged ring in **51** also gave loss of sweetness.

The development of **44** from **1**, through a limited number of deliberately varied structural changes, has resulted in a potentially useful new sweetener.

E. M. ACTON
H. STONE*

Stanford Research Institute, Menlo Park, California 94025

References and Notes

1. E. M. Acton, M. W. Lerom, H. Stone, U.S. Patent 3,919,318, 11 November 1975.
2. S. Furukawa and Z. Tomizawa, *J. Chem. Ind. Tokyo* **23**, 342 (1920).
3. E. M. Acton, H. Stone, M. A. Leaffer, S. M. Oliver, *Experientia* **26**, 473 (1970).
4. E. M. Acton, M. A. Leaffer, S. M. Oliver, H. Stone, *J. Agric. Food Chem.* **18**, 1061 (1970).
5. Taste potency is the inherent taste intensity of a compound, compared to a reference standard, usually sucrose. Comparison is made on water solutions, and potency of the test compound (times the reference) is calculated from the observed taste intensity of its solution and the concentration (*4*, *7*).
6. In Fig. 1, the potency (370 ×) of **1** is revised from our earlier figure (*4*) based on dextrose as reference, and corresponds to 800 × on a weight/weight basis. Probably the figure 2000 × (*2*) was on the basis of weight.
7. The oximes in Fig. 1 were purified and characterized, and water solubilities were determined. A taste panel then compared the oximes in water solution to 0.25*M* sucrose for intensity of the total taste sensation, identified which kinds of tastes (sweet, sour, salty, bitter, other) were present, and quantified these taste qualities as percentages of the total taste (*4*). Such quantification is essential for comparison of compounds, and the relationships observed were reproducible.
8. In following taste-structure relationships, potency (*5*) relative to sucrose is calculated on a mole/mole basis; but ultimately, in the practical application of a sweetener, potency is calculated on a weight/weight basis.
9. All our observations confirm our prediction (*3*) that "α,β-unsaturated aldoximes bearing an alkyl substituent on the α-carbon exist only in the *syn* form." This constitutes a practical advantage over aldoximes existing as both *syn* and *anti* isomers, such as the cyclohexane and benzene analogs of the 1-cyclohexenes or when R_α = H in **2**.
10. E. M. Acton, M. A. Leaffer, H. Stone, U.S. Patent 3,780,194, 18 December 1973.
11. ———, U.S. Patent 3,699,132, 17 October 1972.
12. The epoxide, ketone, and *O*-methyl ketoxime substituents caused acid instability.
13. E. M. Acton, K. Yamamoto, H. Stone, U.S. Patent, 3,833,654, 3 September 1974.
14. L. B. Kier, *J. Pharm. Sci.* **61**, 1394 (1972); H.-D. Höltje and L. B. Kier, *ibid.* **63**, 1722 (1973).
15. Oxime **34** had been described as "unangenehm süss" [A. Eichengrün and A. Einhorn, *Chem. Ber.* **23** (part 2), 2885 (1890)].
16. Reports on initial stages of toxicology studies on **44** (called SRI Oxime V) are in preparation (T. A. Jorgenson, G. W. Newell, D. P. Sasmore, and C. J. Rushbrook, Abstracts of Papers of the Society of Toxicology 15th Annual Meeting, Atlanta, 14 to 18 March 1976, p. 97). In vitro tests of **44** in *Salmonella typhimurium* and *Saccharomyces cerevisiae* showed no evidence of mutagenicity; we are indebted to Dr. V. F. Simmon of the Stanford Research Institute for this test.
17. We thank M. W. Lerom, K. Yamamoto, M. A. Leaffer, C. W. Mosher, and P. A. Sturm for synthesis of oximes and S. M. Oliver for taste panel evaluations.
* Present address: Tragon Corporation, Palo Alto, California 94302.

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