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Synthesis and Olfactory Properties of Some Thiazoles with Bell Pepper Like Odor

Paolo Pelosi, Pierluigi Pasqualetto, and Roberto Lorenzi*

Ten new cycloalkanethiazoles have been synthesized and their olfactory properties studied in relation to 4-butyl-5-propylthiazole. Only derivatives containing eight carbon atoms attached to the thiazole ring have the characteristic bell pepper odor. The study of olfactory thresholds has shown that such odor is most pronounced with 4-isopropyl-7-methylcyclohexathiazole; clear differences, both in odor quality and in olfactory threshold, have been found between stereoisomers of this odorant.

A number of pyrazine and thiazole derivatives exhibit a characteristic bell pepper like odor, associated with extremely low olfactory thresholds. These compounds seem to stimulate a common specific receptor, recently identified in the olfactory mucosa of several mammals (Pelosi et al., 1982). Several authors (Seifert et al., 1970, 1972; Pittet and Hruza, 1974; Parliment and Epstein, 1973; Buttery et al., 1976) have investigated the relationship of bell pepper odor and low olfactory threshold to chemical structure and have recognized common parameters in the molecules of pyrazines and thiazoles with such an odor. It is evident from their results that the lipophile part of the molecule plays an important role in the odor of the compound. However, the odorants examined so far bear hydrocarbon chains of medium length and therefore can adapt to the specific receptor in a great number of different conformations. In order to define with a greater accuracy the shape that the hydrocarbon side of bell pepper odorants must have for best fitting into the olfactory receptor and therefore for eliciting the characteristic odor, we have synthesized 10 new cycloalkanethiazoles that can be regarded as conformational models for 4,5-dialkylthiazoles.

EXPERIMENTAL SECTION

Synthesis of Compounds. All the thiazoles have been prepared according to two general routes, both starting from ketones. Route a involves bromination of the ketone, according to Catch et al. (1948), and cyclization of the bromo ketone with thioformamide, following the method of Kurkjy and Brown (1952). Route b involves direct synthesis of the 2-aminothiazole by reaction of the ketone with thiourea and iodine, according to the procedure of Hurd and Wehrmlister (1949), followed by deamination of the corresponding diazonium salt in the presence of hypophosphorous acid, as described by Roussel and Metzeger (1962). L-4-Isopropyl-7-methylcyclohexathiazole was prepared from L-menthone, obtained by oxidation of natural L-menthol with sodium dichromate, following the procedure reported in Vogel (1957). The optical activity of L-menthone was $[\alpha]^{25}{}_D$ −25.6°, corresponding to an optical purity better than 90%. All the thiazoles were purified by distillation to a grade better than 99.9%, as checked by GLC, by using a 3 mm × 1.5 m column, packed with 5% OV-17 on Anakrom. Mass spectra were recorded on a Hewlett-Packard 5992 B GC–MS, equipped with a jet separator and by using an ionization voltage of 70 eV. Odor threshold were measured in aqueous Tris-HCl, 0.05 M, buffer, at pH 7.0, by using panels of 18–25 subjects, following the method described by Amoore et al. (1968, 1975). Odor quality was judged informally by the authors and few other subjects in the laboratory.

RESULTS AND DISCUSSION

Synthesis of Compounds. Figure 1 shows the structures of the compounds prepared, while the data on their synthesis, structure, and olfactory properties are reported in Table I. Two derivatives, cyclohexathiazole and cyclooctathiazole have been prepared with both methods, to compare yields and purity of the final products. Although, on the average, both routes were equally satisfactory and gave easily purifiable products, the cyclization of the bromo ketones proved difficult in the cases of strained or large rings; in fact, it failed with 2-bromocyclopentanone and 2-bromocyclododecanone and gave poor yield with 2-bromocyclooctanone.

4-Isopropyl-7-methylcyclohexathiazole obtained from commerical menthone, i.e., from a mixture of the four possible stereoisomers, appeared to be constituted by a single pair of enantiomeric compounds, showing a single peak, when gas chromatographed on several different columns (packed OV-1 and OV-17 and capillary OV-1 and FFAP). The retention time is the same as that shown by

Istituto di Industrie Agrarie and Istituto di Orticoltura e Floricoltura, Università di Pisa, 56100 Pisa, Italy.

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Table I. Synthesis, Mass Spectra, and Olfactory Properties of Thiazole Derivatives

thiazole	method of synthesis	yield, %	bp, °C/mmHg	mass spectrum, *m/e*	olfactory threshold, ppb	odor description
4-butyl-5-propyl (I)					0.005	bell pepper
cyclopenta (II)	b	6	54/0.8	*125 (100)*, 124 (71), 97 (48), 98 (23)	550	rubber
cyclohexa (III)	a b	37 25	67/1.0	*139 (100)*, 111 (94), 112 (31)	220	rubber
cycloocta (IV)	a b	4 5	88/1.0	*167 (100)*, 139 (86), 111 (53), 138 (41), 112 (38), 125 (23), 124 (22), 113 (20)	670	rubber/bell pepper
cyclodeca (V)	b	27	112/0.9	*195 (100)*, 152 (72), 162 (64), 111 (53), 112 (48), 113 (43), 166 (40), 138 (31), 134 (30), 125 (23)	580	bell pepper/green
cyclododeca (VI)	b	12	134/0.7	113 (100), *223 (95)*, 180 (84), 126 (60), 166 (51), 112 (47), 190 (45), 140 (37), 182 (33), 111 (32)	700	green/camphor
4-methylcyclohexa (VII)	b	37	76/1.0	138 (100), *153 (62)*, 125 (26), 111 (25), 124 (24)	24	rubber/camphor
6-methylcyclohexa (VIII)	a	12	82/1.0	111 (100), *153 (73)*, 138 (17)	49	disinfectant
4-*tert*-butylcyclohexa (IX)	a	20	86/0.7	139 (100), 138 (68), 140 (12), 111 (12), *195 (11)*	1.5	green/camphor
DL-4-isopropyl-7-methylcyclohexa (X)	a	7	94/1.0	152 (100), 153 (44), *195 (13)*, 125 (13)	0.36	bell pepper
L-4-isopropyl-7-methylcyclohexa (X)	b	22	94/1.0	152 (100), 153 (49), *195 (14)*, 125 (13)	22	green/minty

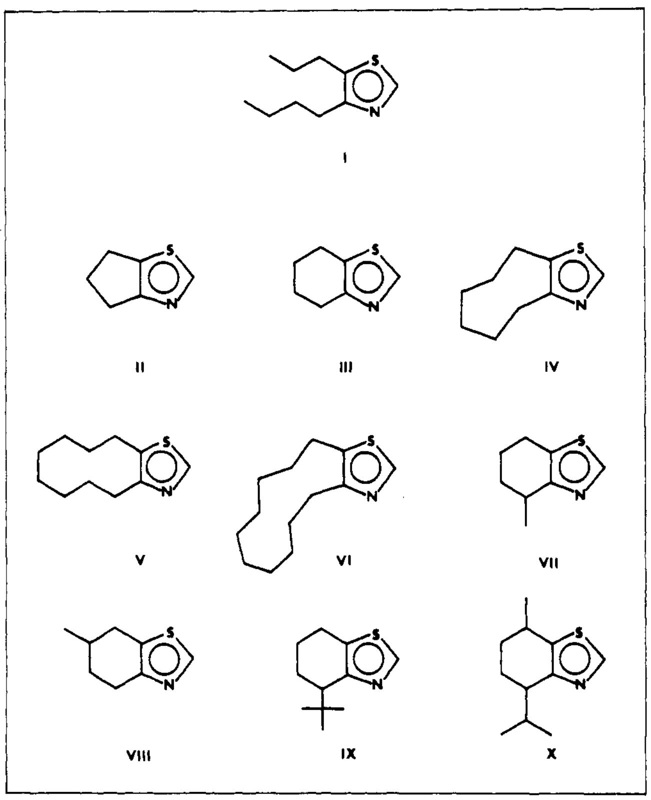

Figure 1. Structures of thiazole derivatives.

L-*trans*-4-isopropyl-7-methylcyclohexathiazole, prepared from natural L-menthol. Furthermore, mass spectra of these two samples were almost exactly superimposable. This leads to the hypothesis, not yet confirmed, that the compound obtained from commercial menthone is only the trans racemate.

As far as the authors can determine, all the componds of Figure 1, except 4-butyl-5-propylthiazole, had not previously been described.

Odor Properties. Table I also reports the odor properties of the thiazoles synthesized, compared to those of 4-butyl-5-propylthiazole. For the cyclohexa- and cyclooctathiazoles, the samples prepared by the two different routes had the same odor quality, but the thresholds were measured in both cases with those obtained by route b.

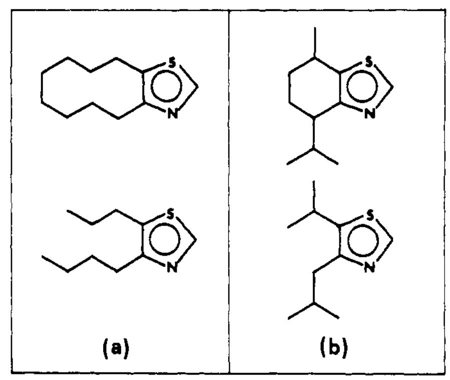

Figure 2. Cyclodecathiazole (a) and 4-isopropyl-7-methylcyclohexathiazole (b) as conformational models for 4-butyl-5-propylthiazole and 4-isobutyl-5-isopropylthiazole.

All the compounds are weaker odorants than the reference derivative, and very few of them exhibit a bell pepper like odor. This behavior has to be expected on the basis that cyclic structures have lower flexibility than open chain derivatives and consequently poorer chances of adapting to the specific receptor site. This confirms also the high specificity of such receptor, not only with regard to the position of the two heteroatoms (Pittet and Hruza, 1974; Buttery et al., 1976) but also with regard to the shape of the hydrophobic side of the molecule. In the series of the unbranched derivatives, from cyclopentathiazole to cyclododecathiazole, the olfactory threshold is rather uniformly high, whereas the bell pepper odor is present, associated with other notes, only in the cyclodeca and cycloocta derivatives. This confirms a size requirement of about seven to eight carbon atoms attached to the thiazole ring. In particular, cyclodecathiazole mimics some of the many conformations of 4-butyl-5-propylthiazole (Figure 2a).

All the other compounds of Table I are derivatives of cyclohexathiazole. A single methyl group attached to the cyclohexane ring is enough to cause a marked increase in odor potency, the effect being more pronounced in the 4-methyl than in the 6-methyl derivative. A more bulky radical, as a *tert*-butyl, in position 4 has a dramatic effect on the threshold, which falls to 1.5 ppb, while the odor becomes more definite. DL-4-Isopropyl-7-methylcyclohexathiazole shows the lowest threshold among the compounds of this series and a well-characterized odor of bell pepper. Surprisingly, we found that the L isomer had a different odor and a much higher threshold. It follows that, if the compound obtained from commercial menthone is free from cis isomers, the bell pepper odor is due only to the enantiomer D-*trans*-4-isopropyl-7-methylcyclohexathiazole; its olfactory threshold then should be 0.18 ppb, more than 100 times lower than that of its enantiomer. This finding, however, needs further investigation. A comparison between the structure of 4-isopropyl-7-methylthiazole and that of 4-isobutyl-5-isopropylthiazole (Figure 2b), which shows an extremely intense odor of bell pepper (Pelosi and Pasqualetto, 1981), shows that the first compound mimics a particular situation among the many conformations of the second derivative and defines with greater accuracy the shape requirements for the hydrophobic part of such odorants, and, consequently, the complementary shape of the corresponding olfactory receptor. We plan to measure binding constants between these odorants and the specific olfactory receptor and to establish correlations between such constants and odor properties.

Registry No. I, 57246-59-0; II, 5661-10-9; III, 4433-49-2; IV, 7140-68-3; V, 84648-03-3; VI, 84648-04-4; (±)-VII, 84648-05-5; (±)-VIII, 84648-06-6; (±)-IX, 84648-07-7; DL-*trans*-X, 84648-08-8; L-*trans*-X, 84710-13-4; D-*trans*-X, 84710-14-5; L-menthone, 14073-97-3; 2-bromocyclooctanone, 39261-18-2.

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Capsaicin Production in Sweet Bell and Pungent Jalapeno Peppers

B. J. Rowland, B. Villalon, and E. E. Burns*

Morphological, histological, and chemical analyses of the interlocular septums of jalapeno peppers and bell peppers were compared. Scanning electron, bright-field, and transmission electron microscopic examinations of the interlocular septums showed little histological difference of the interlocular septa. Glandular regions of oil-producing cells are present in both varieties of peppers. Thin-layer chromatography of the oil produced by pepper gland cells demonstrated that bell peppers produce neutral lipids, glycolipids, and capsaicinoids. Conclusive evidence was found that capsaicin is synthesized in the glandular areas of the interlocular septum of jalapeno peppers.

The jalapeno pepper originated in Jalapa, Mexico, and is becoming increasingly popular with U.S. consumers. One of the more desirable characteristics of jalapeno peppers from a consumer's point of view is pungency, or the ability to produce an organoleptic sensation of heat. The component of jalapeno and other peppers which produces this sensation is capsaicin, a chemical compound that is odorless and flavorless. It is located mainly in the cross walls of hot peppers and spreads throughout the pod during processing. The outer wall of the raw jalapeno has a flavor identical with that of bell pepper (Huffman et al., 1978).

The location of synthesis of capsaicin within the fruit is a subject of much dispute. Newman (1953) felt that the seeds contained little or no capsaicin. Balbaa et al. (1968) published data that indicated the opposite. Both, however, demonstrated that the majority of capsaicin was associated with the dissepiment portion of the fruit. Huffman et al. (1978) confirmed this using gas chromatography. They determined that higher levels of capsaicin are found in the cross walls. Small amounts found in the seed portion was thought to be due to surface contamination during dissection. They examined the cross wall portion of the jalapeno with light microscopy and failed to detect the presence of anatomical structures associated with the capsaicin. However, an intense yellow pigmentation can be observed in both hot and sweet peppers.

Plant glands are a distinct group of highly specialized cells. Glands are composed of secretory cells and often different kinds of auxiliary cells. Different terms are used to denote the type of secretion process. If the secreted material passes directly through the plasmalemma, the process is called eccrine. Material that is transported across the membrane, collected in vesicles, and extruded by exocytosis is called granulocrine secretion. Both forms of secretion are said to be the merocrine type (Schnepf, 1974).

The jalapeno pepper secretes an essential oil that is commonly known as capsaicin, but the type of gland or cell that produces it was unknown prior to this study. the presence of specialized cells which synthesize capsaicin in the cross walls was delineated as well as other aspects of the production of essential oils in jalapenos. Histological

Seafood Technology, Texas A&M University, College Station, Texas 77843 (B.J.R.), Texas A&M University Agricultural Research and Extension Center, Weslaco, Texas 78596 (B.V.), and Adriance Laboratory, Texas A&M University, College Station, Texas 77843 (E.E.B.).

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