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CORRELATION OF THE REACTIVITY OF 1,4-DIALDEHYDES WITH METHYLAMINE IN BIOMIMETIC CONDITIONS TO THEIR HOT TASTE: COVALENT BINDING TO PRIMARY AMINES AS A MOLECULAR MECHANISM IN HOT TASTE RECEPTORS

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Abstract - Examination of selected natural and synthetic 1,4-dialdehydes has shown that hot tasting molecules react with methylamine in biomimetic conditions while their tasteless isomers do not. The *in vitro* reactivity correlates with the biological activity of compounds with comparable molecular shapes and dimensions. The course of the *in vitro* reaction and the nature of the products have been monitored by ^{1}H-NMR, showing that a common charged azomethine intermediate is involved. We suggest that the biological mechanism of hot tasting dialdehydes may include covalent binding to primary amino groups of taste receptors.

INTRODUCTION

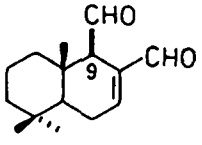
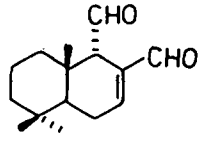
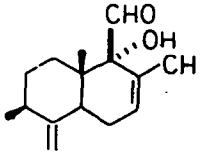
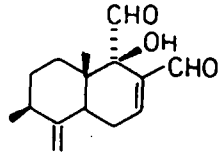
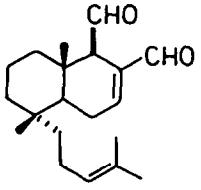
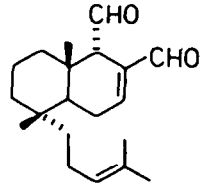
One of the major values of biochemical studies in taste receptor lies in gaining insight into the relationship between molecular events and taste function.

The hypothesis of "receptor proteins" in taste has been addressed experimentally by only a few laboratories and remains poorly understood[2]. Several limitations pose significant problems in exploring taste receptors at the molecular level. One in particular,the paucity of high affinity,high specificity receptor ligands, seems critical for distinguishing isolated receptor membranes from membranes associated with adiacent cells[3]. Therefore there is a general need for reactive ligands that specifically interact with particular taste receptor sites and are capable of covalently bonding to the sites.The use of such receptor probes would be of great value in enabling isolation and identification of receptor macromolecules.

Circumstantial evidence suggest that the hot taste for the human tongue as well as the antifeedant activity towards insects displayed by several naturally occurring 1,4-dialdehydes[4] could be related to the formation of covalent bonds with the receptors in the course of taste detection. Electrophysiological studies have shown that when the maxillary palp (equivalent to taste buds) of the *Spodoptera exempta* larva are repeatdly contacted with filter paper impregnated with warburganal (*17*),one of the above mentioned dialdehydes,the taste sense is irreversibly blocked[5]. A practical consequence is that when the armyworm is placed on a maize leaf topically treated with warburganal and subsequently transferred to an untreated leaf,it starves to death[5].

One striking feature of these molecules is that their persistent hot taste for the human tongue,which parallels their feeding inhibition of animals[4],is strictly dependent on the distance between the two aldehyde groups. Table I lists three pairs of these dialdehydes,differing only in the stereochemistry of the C-9 aldehyde group, exemplifying this behaviour. The 9β-dialdehydes polygodial (*1*),muzigadial (*3*) and saccalutal (*5*) are all hot tasting while their 9α-isomers *2*,*4* and *6* are reported to

Table I. Taste for the human tongue of 9ß,9α 1,4-dialdehyde pairs.

Hot tasting dialdehydes	Tasteless dialdehydes
 *1*[4,6]	 *2*[4,6]
 *3*[7]	 *4*[7]
 *5*[8]	 *6*[8]

[a] Literature cited refers to the biological activity.

be tasteless.

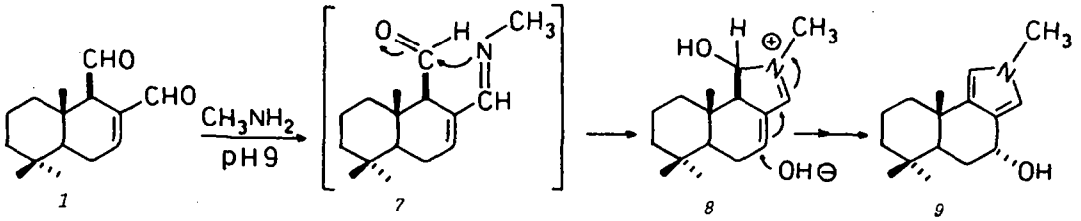
An interpretation[9,10] of the behaviour for the *1,2* pair, based on the different reactivity observed for *9*ß-or *9*α-polygodial towards primary amines in biomimetic conditions, suggest that the biological activity of the *9*ß-dialdehyde might be due to the formation of covalent bonds with primary amino groups *in vivo*.

This paper reports additional evidence supporting the above hypothesis.

RESULTS AND DISCUSSION

Previous work with the polygodial (*1*) - isopolygodial (*2*) pair has shown that *1* is capable of reacting with methylamine in "biomimetic" conditions (phosphate buffer, pH 9 - CH_3CN) while its tasteless isomer *2* is unreactive under the same conditions[9].

When the reaction of *1* with methylamine was monitored by ^{1}H-NMR[10], the formation of the crucial intermediate *8* was evidenced which, in turn, slowly evolved to the pyrrole derivative *9* (*Scheme 1*).



Scheme 1

The formation of *8* clearly depends upon the distance between the carbonyl carbon and the nitrogen atom in the hypothetical intermediate *7*;that is,upon the distance between the two aldehyde groups. *1*,which displays the shorter distance (2.7 Å in the intermediate *7*;Dreiding models) is reactive,while *2* (3.0 Å in the the intermediate analogous to *7*) does not react.

Since the formation of type *8* adducts in a non-enzymatic reaction with primary amino groups *in vivo* is a highly probable event,our working hypothesis was that the minimum requirement for exhibiting a hot taste is the capability of a 1,4-dialdehyde in forming type *8* adducts.

For proving (or disproving) the above hypothesis we devised to establish a parallel between the ability in reacting with methylamine in biomimetic conditions and the hot taste of a group of selected 1,4-dialdehydes.

Since type *8* adducts are non-isolable compounds[10] and the hydroxy-pyrrole derivatives are highly unstable[9] the reactions with methylamine were followed by ^{1}H-NMR without isolation of the products and therefore the information on the course of the reactions rests only on this technique and on the evidence coming from previous work[9,10].

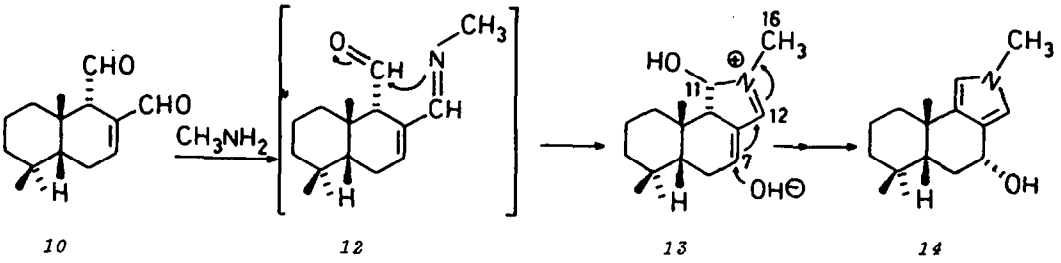
The general conditions of the reaction were the following. The dialdehyde (ca. 2 mg),dissolved in CD_3CN (0.5 mL),was added to a solution of $Na_2HPO_4 \cdot 12H_2O$ (28.6 mg) in D_2O (4 mL) containing CD_3CN (1 mL). When the mixture was not clear the amount of CD_3CN was slightly increased and finally $CH_3NH_2 \cdot HCl$ (3 mg) was added. Due to the high dilution and to the complexity of the solutions,monitoring was satisfactory only with a 500 MHz spectrometer. Even with this powerful machine, the poor resolution did not enable us in many istances to exactly measure the coupling constants.

An interesting pair of C-9 isomeric dialdehydes is constituted by *10* and *11*, which are synthetic products[11,4],differing from the *1-2* pair for the cis ring junction.

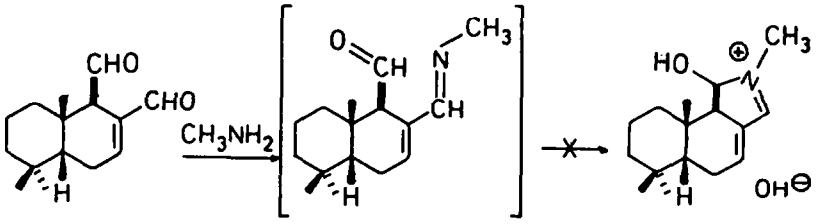
Compound *11* is tasteless and unreactive with CH_3NH_2 in biomimetic conditions, while the hot tasting compound *10* shows a trend of reactivity similar to that of polygodial (*1*).

In a ^{1}H-NMR spectrum taken 10 min after the addition of CH_3NH_2 were observed signals consistent with the presence into the solution of both *13* and *14*[12] in a 1:3 approximate ratio,besides the presence of the starting compound *10* (ca. 50%). Signals at δ 8.57,7.16,5.63 and 3.48 were attributed to the protons at C-12,C-7, C-11 and C-16 respectively of the intermediate *13* by analogy with the corresponding resonances displayed by compound *8*[10]. After 2.5 h the spectrum contained only signals due to the presence of *14* (Experimental).

Difference in reactivity between *10* and *11* was explained on consideration of the difference of the distances[13] between the nitrogen atom and the carbonyl carbon in the hypothetical intermediate *12* (2.3 Å) and *15* (3.2 Å): apparently only the shorter distance allows the formation of the charged azomethine intermediate *13* while *11* was found unchanged by ^{1}H-NMR monitoring even two days after the addition of CH_3NH_2.



10 *12* *13* *14*



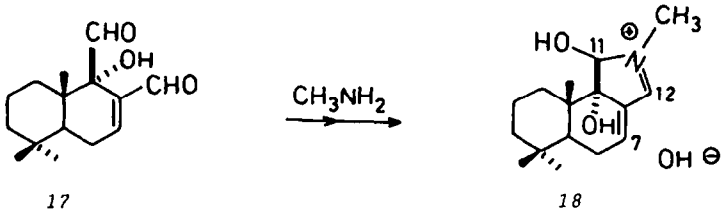
11 *15* *16*

Thus the *10-11* pair parallels the behaviour of the *1-2* pair both in the *in vitro* reactivity and in the biological activity, in the sense that only the dialdehyde with a suitable distance between the two -CHO groups may react *in vivo* with primary amino groups and, hence, display biological activity.

In principle the same considerations applie to the *3-4* and *5-6* pairs in which the biological activity was found only in the compounds (Table I) displaying the shorter distance[14] between the two -CHO groups (*3* and *5*).

While *5* should have the same *in vitro* reactivity as *1*, it is expected that the presence of an additional -OH group in *3* could have consequence on the chemical reactivity. Since *3* was not available, the reactivity of such a type of compound was tested on warburganal (*17*) the most active compound in the series[4-6].

17, synthesized by known procedures[15], smoothly reacts with methylamine in the usual conditions affording a compound for which ^{1}H-NMR data (Fig.1) strongly suggest structure *18*. The C-12, C-7 and C-11 protons were observed at δ 8.50, 7.26 and 5.62 respectively, while the N-methyl protons resonated at δ 3.47. Compound *18* does not evolve to pyrrole derivatives and is observed in solution even 48 h after the initial mixing of the reactives.



17 *18*

The reactivity of warburganal exemplifies the importance of the charged azomethine moiety (e.g. *8,13,18*) in assessing a parallel between the *in vitro* reactivity and the hot taste: in the conjecture that the *in vitro* reactivity could be assimilated to an *in vivo* non-enzymatic reaction, the charged azomethines (and not the pyrrole derivatives) are the sole compounds which are smoothly formed and are common to all

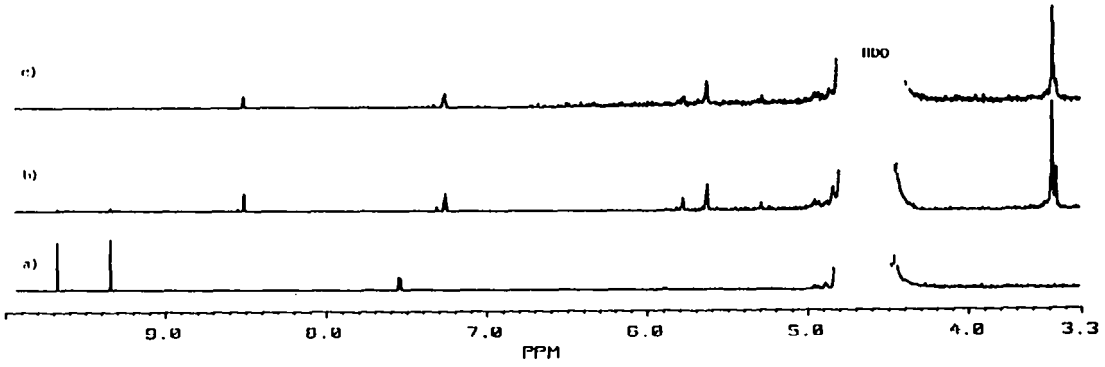


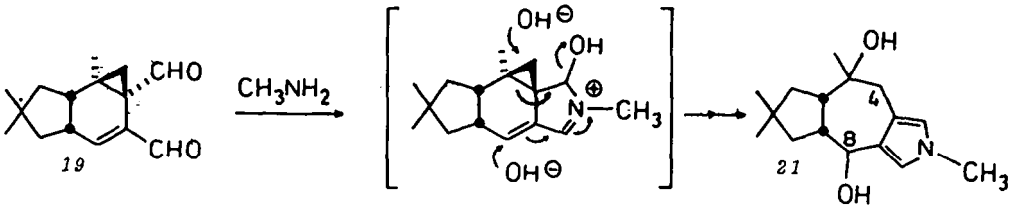
Fig.1. ^{1}H-NMR spectra (3.3-10 ppm region) of a) warburganal(*17*;phosphate buffer, pD 9); b) 5 min after addition of $CH_3NH_2 \cdot HCl$; c) 180 min after addition of $CH_3NH_2 \cdot HCl$.

the dialdehydes considered . In this respect these species could be considered to be primarily involved in the biological response to the human tongue.

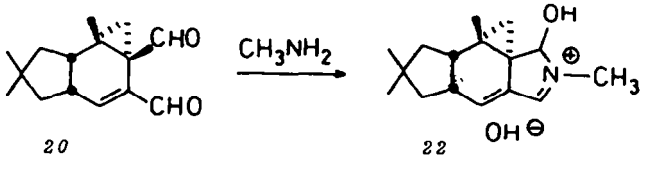
Confirmation that the biological activity is strictly dependent from the distance between the two aldehyde groups comes from the examination of the *19-20* pair. Isoverrelal (*19*) is a pungent molecule isolated from fungi [16] while its isomer, iso-isovelleral (*20*),is a synthetic compound[17]. Because of the cis ring junction, in compound *19* the distance we are dealing about is depending on the conformation in solution and was found to range from 2.8 to 3.2 Å on Dreiding models. Interesstingly,the same distances into iso-isovelleral (*20*) ranged from 2.6 to 2.9 Å, allowing us to predict [17] that also *20* would taste hot to the human tongue. Tasting of *20* in the usual manner[4] confirmed the hypothesis.

The fact that both isomers *19* and *20* exhibit an hot taste indicates that concevaibly in these medium sized molecules the distance between the two aldehyde groups is the only critical prerequisite for displaying the biological activity.

Isovelleral (*19*) reacts very slowly with methylamine in the usual conditions. A reaction product starts to appear after three days and has been formulated as *21* on the following evidence.



Scheme 2



The ^{1}H-NMR spectrum of the mixture shows signals at δ 6.67 and 6.51 which were attributed to pyrrole protons. Since formation of the pyrrole ring implies opening of the cyclopropane,it was postulated that the usual azomethine intermediate (*Scheme 2*) suffers two OH^- attacks leading to *21*. The ^{1}H-NMR spectrum supports structure *21*: the newly formed methylene (C-4) resonates as an isolated AB quartet at δ 3.02 and 2.74 (J 18.2 Hz),the methyl group geminal with the tertiary alcohol was found at δ 1.19,while the C*H*OH proton (C-8) resonates as a doublet (J 3.2 Hz) at δ 4.08. In the *in vitro* conditions selected for the reaction the starter product disappeared slowly: *21* became the sole compound in the reaction mixture only after 21 days.

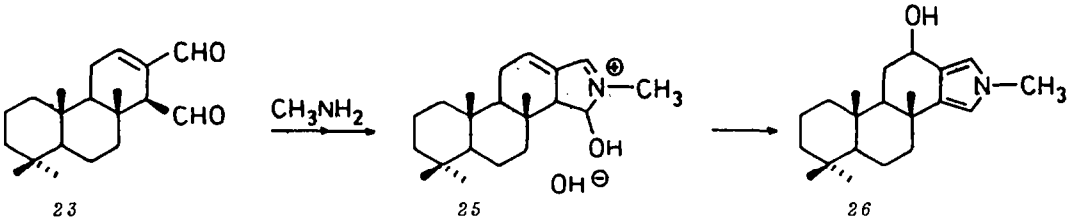
Iso-isovelleral (*20*) parallels isovelleral in reacting very slowly with CH_3NH_2. Monitoring of the reaction after seven days showed small signals (ca.10% of the signals of the starter product) in the ^{1}H-NMR spectrum at δ 8.40,7.35, 5.07 and 3.56 which were assigned to the presence of the intermediate *22*. Further monitoring at longer times showed that neither *20* or *22*,nor other products were present in the solution.

We have already reported[4] that tricyclic and tetracyclic dialdehydes such as *23* and *31* are tasteless while possessing the same distance between the two aldehyde groups as the hot tasting molecules *1*,*3* and *5*. 12-Epi-scalaradial (*27*)[18] also belongs to these tasteless molecules.

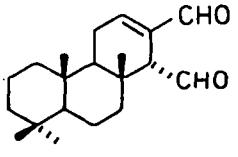
Although such a behaviour could also be accounted for otherwise,an intriguing hypothesis might be that these molecules cannot fit the appropriate receptor sites because of their bulk.

The isomers *24*[19] and *28*[18],displaying in addition a major distance between the aldehyde groups,were found (more expectedly) tasteless.

The chemical reactivity of the *23*-*24* and *27*-*28* pairs was the expected one: *23* and *27* react with methylamine while *24* and *28* are unreactive under the same conditions. In Fig.2 representative spectra of the reaction of *23* with CH_3NH_2 are reported. The observed chemical shift values,very close to those found in the reaction mixtures of *1* and *10*,suggest structure *25* for the intermediate azomethine and *26* for the final product. Similarly, *27* affords *29* and *30* (Experimental).



23 *25* *26*



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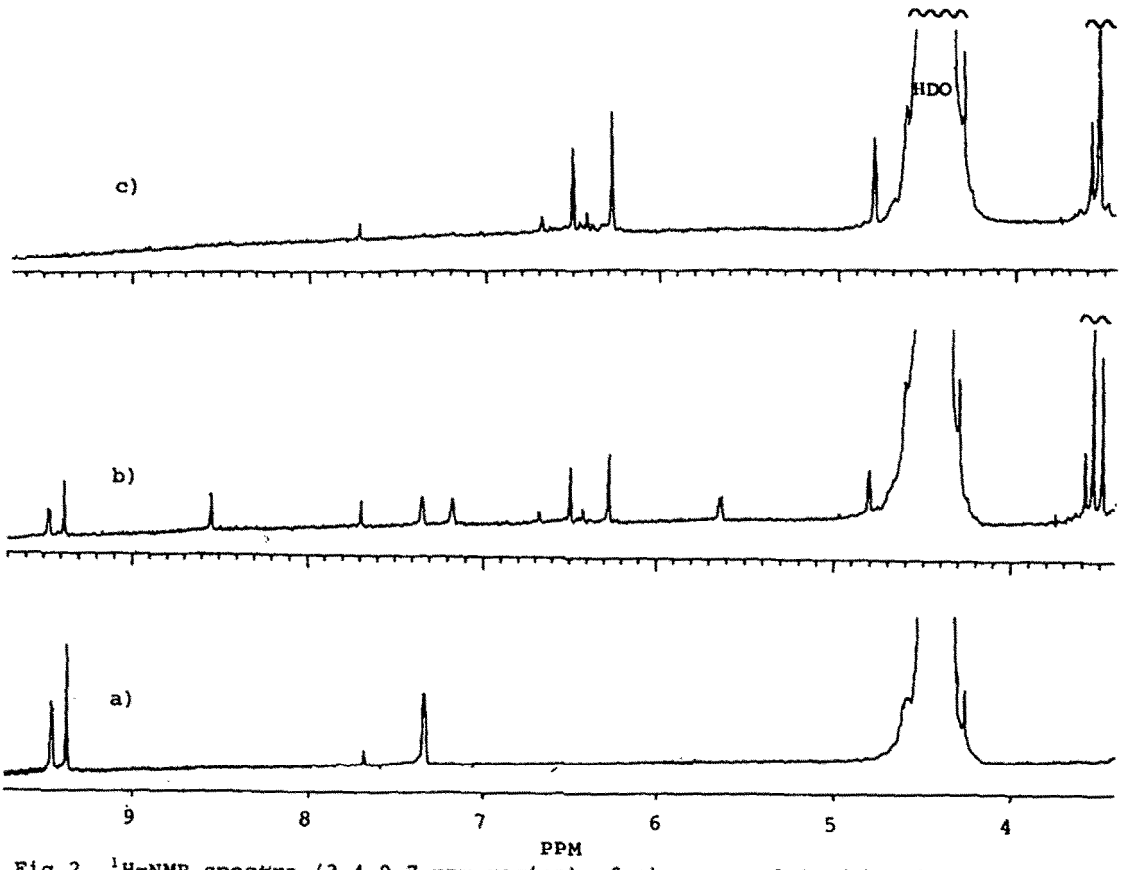
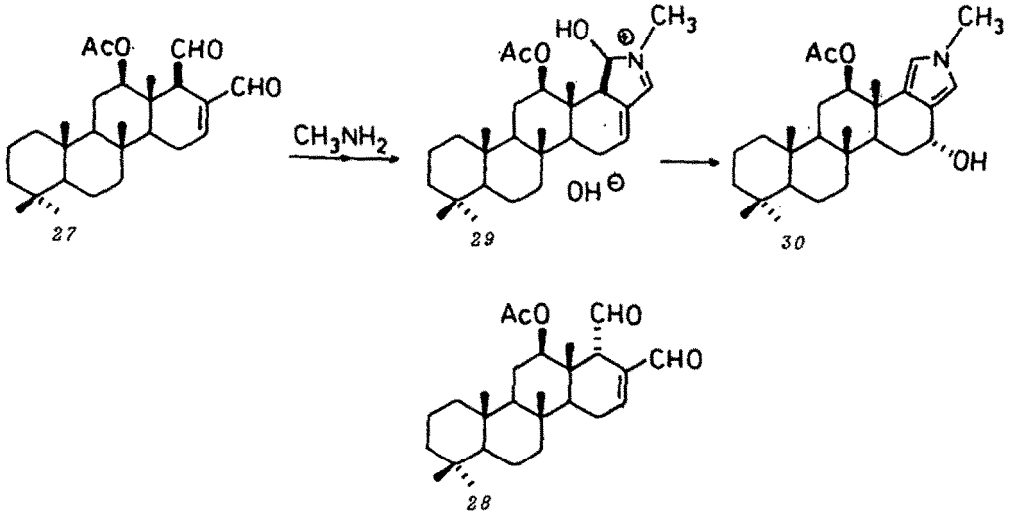


Fig.2. ^{1}H-NMR spectra (3.4-9.7 ppm region) of a) compound *23* (phosphate buffer, pD 9); b) 5 min after addition of $CH_3NH_2 \cdot HCl$; c) 120 min after addition of $CH_3NH_2 \cdot HCl$.

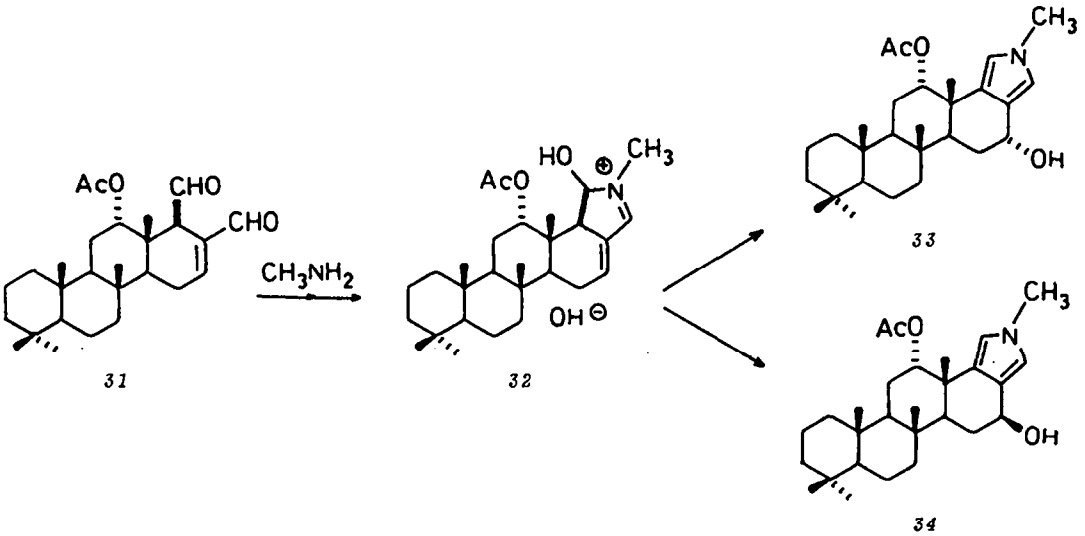


The mixture of the reaction of scalaradial (*31*) with CH_3NH_2 is more complex. Monitoring of the reaction by ^{1}H-NMR after 20 min revealed the presence of signals at δ 8.55,7.13,5.63 and 3.45 attributable to the intermediate *32*,while after 24 h the spectrum indicated the presence of three products since three -NCH_3 signals were found resonating at δ 3.55,3.51 and 3.50.

Structures *33* and *34* were attributed to the two major products (ca. 1:1) on the following evidence. The four signals at δ 6.67,6.48,6.21 and 6.06 were attri-

buted to pyrrole protons while the signals at δ 5.25 and 5.20 were assigned to the -C*H*OH protons in *33* and *34*. Pyrrole protons at δ 6.48 and 6.06 show chemical shift values close to those usually found in the reaction mixtures of the related dialdehydes and should be due to the compound *33* in which the OH^- attack has taken place from the less hindered face of the molecule. The other two signals were assigned to the compound *34* in which OH^- attack has taken place from the β face of the molecule.

The unusual behaviour of *31* is probably due to the presence of the 12α-acethoxy functionality which hinders the α face of the molecule and allows the OH^- attack on the intermediate *32* from both faces of the molecule[20].

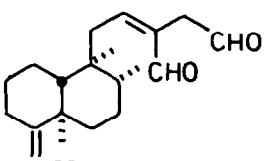


31 *32* *33* *34*

CONCLUSION

It is reasonable to suppose that tasting employs a variety of receptor sites; some of these may bind tasting molecules covalently. Likely candidates for covalent binding would include the hot tasting dialdehydes discussed here because of their relevant reactivity in biomimetic conditions.

Chemical reactivity with methylamine parallels the hot taste to the human tongue for the bicyclic dialdehydes having a suitable distance between the aldehyde groups. Molecules of major size (*23,27,31*) are tasteless while exhibiting similar reactivity. This fact suggest that only smaller molecules can enter the receptor sites. Obviously molecules having the 1,4-dialdehyde functionality in an acyclic environment,such as isolinaridial (*35*)[21],are also hot tasting since can easily reach the receptor site.



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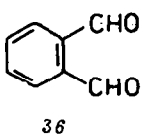

36

Reactivity of the dialdehydes with primary amino groups in the receptor sites explains the observed bioactivity-structure relationship more satisfactorily than the previous suggestion[21,6] of reactivity of the enal moiety with -SH groups.

In this respect,it should be added to the previous objections[9] that phtalaldehyde (*36*),which cannot react with -SH groups like the enal-containing compounds,is also a hot tasting molecule.

EXPERIMENTAL

NMR spectra were recorded on a Bruker WM 500 spectrometer using DSS as internal standard. Dialdehydes *10,11* and *17* were synthesized following literature procedures as previously reported[4]. Dialdehydes *23,24,27,28* and *31* were isolated from natural sources as reported in the appropriate references. Isovelleral (*19*) and iso-isovelleral (*20*) were kindly provided by Dr. O. Sterner (Lund University), while isolinaridial (*35*) was kindly provided by Dr. A. San Feliciano (University of Salamanca). Taste to the human tongue was assayed as previously reported[4].

Reaction of the dialdehydes with CH_3NH_2.

The general conditions of reaction of the dialdehydes with CH_3NH_2 were those reported in the Results and Discussion section,unless otherwise stated. Reactions of the compounds *11,24* and *28* were stopped after 48 h,since no products were detectable by ^{1}H-NMR monitoring.

Reaction of compound *10* with CH_3NH_2.

Standard conditions. ^{1}H-NMR spectra were taken after 10 min and 2.5 h. ^{1}H-NMR monitoring after 2.5 h: product *14*,δ 6.56,6.35,4.97;methyl singlets at δ 3.56,1.25,0.98 and 0.59.

Reaction of warburganal (*17*) with CH_3NH_2.

Standard conditions. ^{1}H-NMR spectra were taken after 2 and 20 min and after 3 and 48 h. ^{1}H-NMR monitoring after 3 h: product *18*,δ 8.50,7.26,5.62;methyl singlets at δ 3.47,0.92,0.90 and 0.72.

Reaction of isovelleral (*19*) with CH_3NH_2.

Standard conditions. ^{1}H-NMR spectra were taken after 10 min,3 h,3,7,17 and 21 days. ^{1}H-NMR monitoring after 21 days: product *21*,δ 6.67,6.51,4.08 (d,J 3.2 Hz) 3.02 (d,J 18.2 Hz),2.74 (d,J 18.2 Hz);methyl singlets at δ 3.51,1.19,0.92 and 0.89.

Reaction of iso-isovelleral (*20*) with CH_3NH_2.

Standard conditions. ^{1}H-NMR spectra were taken after 2 min,3 h,1,2,3,7,17 and 21 days. ^{1}H-NMR monitoring after 7 days,see Results and Discussion.

Reaction of compound *23* with CH_3NH_2.

23 (2.5 mg),dissolved in CD_3CN (0.5 mL),was added to a solution of $Na_2HPO_4 \cdot 12H_2O$ (28.6 mg) in D_2O (4 mL) containing CD_3CN (2 mL). $CH_3NH_2 \cdot HCl$ (3 mg) was finally added to the solution. ^{1}H-NMR spectra were taken after 2,5 and 120 min. ^{1}H-NMR monitoring after 120 min: product *26*,δ 6.51,6.28,4.80;methyl singlets at δ 3.54,1.09,0.90,0.87 and 0.85.

Reaction of 12-epi-scalaradial (*27*) with CH_3NH_2.

27 (2 mg),dissolved in CD_3CN (0.5 mL),was added to a solution of $Na_2HPO_4 \cdot 12H_2O$ (28.6 mg) in D_2O (2 mL) containing CD_3CN (1.5 mL).$CH_3NH_2 \cdot HCl$ (1.5 mg) was finally added to the solution. ^{1}H-NMR spectra were taken after 5,25 and 40 min. ^{1}H-NMR monitoring after 5 min: compound *29*,δ 8.54,7.27 5.72 and 3.48 ($-NCH_3$). ^{1}H-NMR monitoring after 40 min: compound *30*,δ 6.49,6.18,4.73 and 3.53 ($-NCH_3$).

Reaction of scalaradial (*31*) with CH_3NH_2.

31 (3.8 mg),dissolved in CD_3CN (0.5 mL),was added to a solution of $Na_2HPO_4 \cdot 12H_2O$ (28.6 mg) in D_2O (4 mL) containing CD_3CN (3 mL). $CH_3NH_2 \cdot HCl$ was finally added to the solution. ^{1}H-NMR spectra were taken after 5,20,75 and 120 min and after 24 h. ^{1}H-NMR monitoring after 24 h,see Results and Discussion.

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