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REACTION OF POLYGODIAL WITH PRIMARY AMINES: AN ALTERNATIVE EXPLANATION TO THE ANTIFEEDANT ACTIVITY[1]

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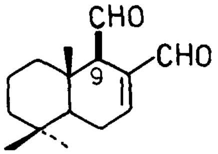
Abstract: *In vitro experiments suggest that the biological activity of polygodial (1) is primarily related to its ability to form adducts with -NH_2 groups rather than -SH groups, as previously reported.*

Polygodial (1) is a sesquiterpene of plant origin[2] which recently has also been isolated from the defense secretion of the Mediterranean nudibranch Dendrodoris limbata[3]. 1,as well as some related dialdehydes,exhibits a number of interesting biological properties,including a marked antifeedant activity against insects[4] and fish[3],and tastes very hot[5] to humans.

However,in spite of various efforts made in recent years,the biochemical mechanism accounting for these activities remains still obscure.

Since the antifeedant activity of polygodial is suppressed when tested in vivo with equimolar quantities of L-cysteine[6],it has been suggested[7] that a reaction of the enal moiety of 1 with -SH groups on the receptors could account for the biological activity of this compound.In line with this view,recently Kubo and Ganjian[8] have shown that under biomimetic conditions cysteine does react with polygodial.However,apart from some kinetic data,the nature of the reaction was not investigated.

For this and other reasons we were doubtful about the proposed mechanism, inter alia because i) it does not convincingly explain the biological inactivity[6] of 9α-polygodial (2) which is also tasteless to humans[8],and ii)of the particular type of the model thiol used,i.e. cysteine,which has two nucleophilic groups (-NH_2 and -SH) capable of reacting with 1.In addition it is known that cysteine readily reacts with aldehydes to form thiazolidine adducts[9].



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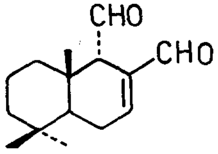

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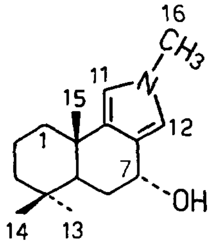

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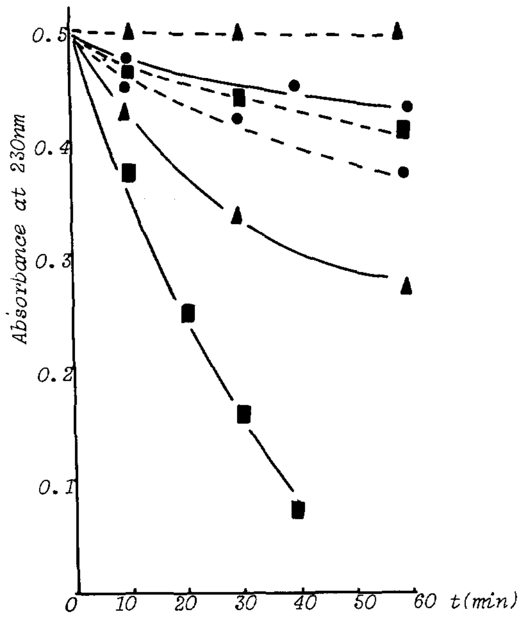

Fig.1.Reactivity and change in absorbance of polygodial (4x10^{-5}M)—— and 9α-polygodial (4x10^{-5}M)--- at 230 nm,with 4x10^{-4} cysteine (■),4x10^{-4} glutathione (▲) and 4x10^{-4} 2-hydroxy-1-ethanethiol (●) in phosphate buffer (pH 9).

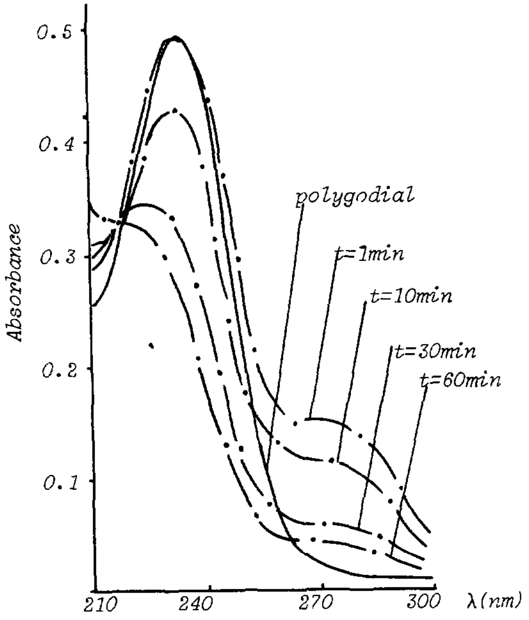

Fig. 2. Absorption spectra of polygodial (4x10^{-5}M) during the reaction with $CH_3NH_2.HCl$ (4x10^{-4} M) in 0.1 M phosphate buffer (pH 9).A similar behaviour was observed with L-lysine and β-alanine.

The absorption spectrum of 9α-polygodial does not change in the same conditions.

We therefore investigated separately the reactivity of 9β- and 9α-polygodial with thiols and primary amines by monitoring the spectral changes which occurr under the reported biomimetic conditions[8].

It can be seen from the data shown in Fig. 1 that thiols,such as 2-hydroxy-1-ethanethiol,display a poor and similar reactivity with both 1 and 2,while the reaction with substrates possessing both -SH and -NH_2 groups is dependent upon the stereochemistry of the 9-CHO group,the 9β-isomer exhibiting the higher reactivity.

A similar trend of reactivity is observed with amines or aminoacids (Fig. 2), the 9α-isomer being practically unreactive.

Taken together,all these data indicate a marked preference of the biologically active 9β-isomer to react with -NH_2 rather than -SH groups.

On these basis,the reaction of 1 with a model amine,i.e. methylamine,was carefully investigated.The reaction,carried out in phosphate buffer at pH 9,afforded a single ether soluble product which owing to its instability was analyzed directly by n.m.r. spectroscopy without further purification.

The relevant p.m.r. data ($CDCl_3$) suggested structure 3 for the reaction product.Two signals at δ 6.53 (1H,d,J 2.1Hz) and 6.22 (1H,d,J 2.1Hz) indicated the presence of a β,β-disubstituted pyrrole ring in the molecule,while both the chemical shift and the coupling constant of a signal at δ 4.89 (1H,bt,J 3Hz) suggested the presence of an axial alcoholic function at C-7.In addition,the spectrum showed four methyl singlets at δ 3.43 (N-CH_3),1.08,0.92 and 0.87.

The c.m.r. spectrum[10] confirmed the proposed structure.In particular,the resonance at δ 63.4 (d) is consistent with the presence of an OH group at C-7.

The detailed mechanism of the reaction of polygodial and related dialdehydes with methylamine in the reported conditions deserves further investigation.

However the observed difference in the reactivity of 9β- and 9α-polygodial towards primary amines could explain the difference in the biological activity: the tasteless 9α-isomer is unreactive owing to its inability to cyclize to pyrrole derivatives.

This finding suggests that -NH_2 groups (e.g. ε-amino group of lysine) rather than -SH groups on the receptors are involved in the taste sense.

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REFERENCES AND NOTES

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