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INSECT ANTIFEEDANT ACTIVITY AND HOT TASTE FOR HUMANS OF SELECTED NATURAL AND SYNTHETIC 1,4-DIALDEHYDES[1]

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ABSTRACT.—The antifeedant activity towards larvae of *Leptinotarsa decemlineata* and *Spodoptera littoralis* and the hot taste for the human tongue have been determined for natural and synthetic 1,4-dialdehydes **1a, 1b, 2, 3, 6, 7, 8,** and **9**. Among the bicyclic dialdehydes the biological activity has been found to be dependent on the distance between the two aldehyde groups; tricyclic and pentacyclic dialdehydes are inactive in both tests. A short synthetic route to (−)- and (+)-polygodial [**1a, 1b**] is reported.

Some naturally occurring sesquiterpenoid 1,4-dialdehydes, such as polygodial [**1a**] and warburganal [**3**], possess potent antifeedant activity against African army worms (1). In addition, they are powerful helicocides (1), inhibit the feeding of fish (2), and kill them (3).

One more striking feature of these molecules is their hot taste (4) for the human tongue, which parallels their feeding inhibition of animals. These properties, however, are strictly related to the stereochemistry of the C-9 aldehyde group. For instance, in contrast with the bioactivities displayed by polygodial [**1a**], the 9α-isomer isopolygodial [**2**] is tasteless for humans and devoid of antifeedant activity toward insects (1) and fish (2); moreover, saccalutal [**4**] kills fish at 0.4 ppm, while isosaccalutal [**5**] does not, even at a concentration of 10,000 ppm (3).

An interpretation (5,6) of this behavior, based on the different reactivity observed for 9β- or 9α-polygodial toward primary amines in biomimetic conditions, suggests that the biological activity of the 9β-dialdehyde might be due to the formation of covalent bonds with primary amino groups in vivo.

In the present paper we wish to report the results of the antifeedant activity tests of dialdehydes selected with a view to obtaining more information on the structure-activity relationship.

The pair of C-9 isomeric dialdehydes with the A/B *cis* ring junction [**6** and **7**], previously synthesized by Guillerm *et al.* (17), was chosen for comparison with the polygodial [**1a**]-isopolygodial [**2**] pair. (+)-Polygodial [**1b**] was synthesized[3] and tested to ascertain whether the absolute configuration, opposite to the natural (−)-isomer [**1a**], might have effects on the biological activity. Finally, two naturally occurring dialdehydes of marine origin [**8** and **9**] having diterpenoidic and sesterterpenoidic skeletons, respectively, were also tested. All compounds were also qualitatively assayed for their taste to the human tongue, in order to make a comparison with the antifeedant activity against insects.

RESULTS AND DISCUSSION

The results of the bioassays are reported in Table 1. The testing of (−)-polygodial [**1a**], isopolygodial [**2**], and warburganal [**3**] confirms the known properties of these

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[1]This work was presented by G. Sodano as a part of an oral communication at the 5th International Symposium on Marine Natural Products, Paris, September 2-6, 1985.

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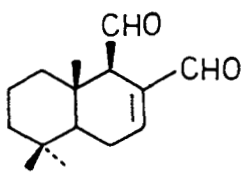
[3]While this account was in preparation, a very complex synthesis of (+)-polygodial has been published (12).



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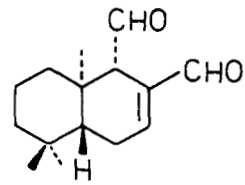

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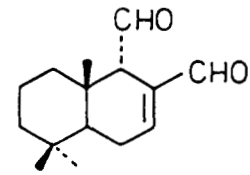

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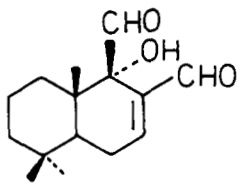

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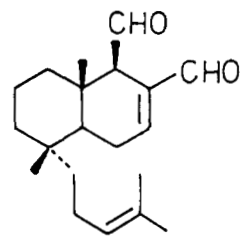

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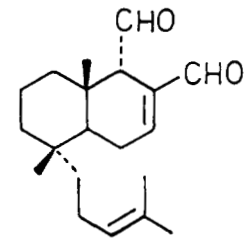

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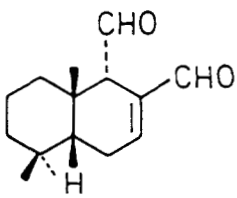

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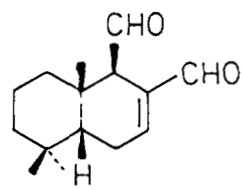

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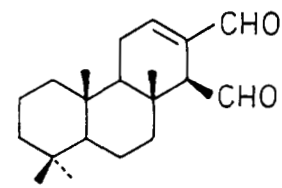

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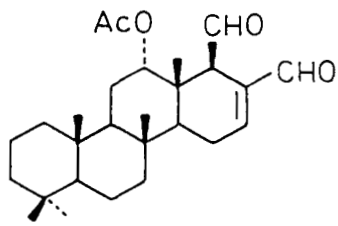

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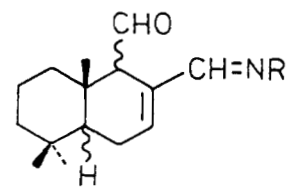

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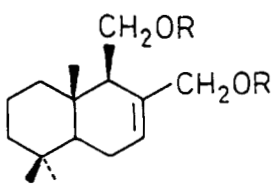
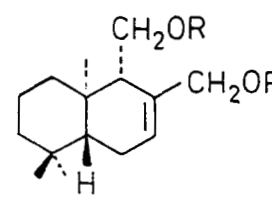


11 R=H
13 R=menthoxyacetyl



12 R=H
14 R=menthoxyacetyl

compounds: polygodial and warburganal are active, warburganal being the most powerful, while isopolygodial is inactive.

(+)-Polygodial [**1b**] is as active as the natural (−)-isomer, showing that the opposite absolute configuration does not have major effects on bioactivity.

The results exhibited by the pair of **6** and **7** having the A/B *cis* ring junction are very interesting to understand the mode of action of these compounds. The 9α-isomer **6** is active on both test insects, while the 9β-isomer **7** displays a modest activity only on *Spodoptera littoralis*. In addition, **6** has a hot taste for the human tongue, while **7** is taste-

TABLE 1. Antifeedant Activity and Hot Taste for Humans of Dialdehydes **1a**, **1b**, **2**, **3**, **6**, **7**, **8**, and **9**

Dialdehyde	Concentration	Antifedant activity				Taste for the human tongue
		Leptinotarsa decemlineata		*Spodoptera littoralis*		
		Choice Test	No Choice Test	Choice Test	No Choice Test	
	100	93	78	97	69	
	50	90	84	92	88	
1a	10	80	66	93	57	hot
	5	72	49	75	19	
	1	48	21	28	2	
	100	86	39	91	76	
	50	49	18	74	41	
1b	10	23	0	55	28	hot
	5	7	4	30	0	
	1	3	2	12	0	
	100	0	0	0	11	
(±)-**2**	50	0	0	0	7	tasteless
	10	0	0	0	0	
	100	94	83	93	90	
	50	88	84	97	79	
(±)-**3**	10	89	80	95	66	hot
	5	81	69	91	45	
	1	70	42	82	24	
	100	40	38	80	56	
(±)-**6**	50	18	11	59	22	hot
	10	0	6	36	4	
	100	9	5	49	18	
(±)-**7**	50	4	0	31	0	tasteless
	10	0	0	7	3	
	100	0	0	19	17	
8	50	0	0	0	0	tasteless
	10	0	0	0	0	
	100	0	0	3	10	
9	50	0	0	0	0	tasteless
	10	0	0	0	0	

less. If these results are compared with those of the polygodial-isopolygodial pair, there is an apparent inversion of activity between the 9β and the 9α stereochemistry in each pair of compounds. This inversion, however, is only apparent. If one considers the distance between the nitrogen and the carbonyl carbon in the hypothetical intermediate **10** arising from interaction with primary amino groups in vivo (6), it can be noticed (Table 2) that in each pair the shorter distance is found in the bioactive compound.[4]

[4]As pointed out earlier in the case of polygodial (6) the intermediate **10** evolves in vitro to a pyrrole derivative through a charged azomethine intermediate whose formation is strictly dependent on the distance of the 9-aldehyde function from the imine nitrogen atom.

TABLE 2. Distances Measured on Dreiding Stereomodels Between the Carbonyl Carbon and the Imine Nitrogen in the Hypothetical Intermediate **10** coming from **1a, 2, 6,** and **7**

	Intermediate **10** from:			
	1a	**2**	**6**[a]	**7**[a]
Distances (Å)	2.7	3.0	2.3	3.2

[a]Steroid-like conformations were considered (10 β-axial; 5 β-equatorial).

Thus, as in the case of the polygodial-isopolygodial pair (5,6), it can be concluded that, in the **6-7** pair, 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. As a confirmation, when **6** and **7** were treated with methylamine under the same biomimetic conditions (5), **6** was found to be reactive, while **7** was recovered unchanged, thus paralleling the behavior of **2**.[5]

The results of the tests with the dialdehydes **8** and **9** were surprising. On the basis of their structural features both compounds would be expected to taste hot to the human tongue and to have antifeedant activity, inasmuch as the relative position of their two aldehyde groups is the same as in polygodial. However, **8** and **9** were tasteless and inactive toward insects. Although such a behavior could also be accounted for otherwise, an intriguing hypothesis might be that these molecules cannot fit the appropriate receptor sites because of their bulk.

In conclusion, the results of this work are in good agreement with the previously known data on the structure-activity relationship of bicyclic dialdehydes. Because the parallel tendency between hot taste for the human tongue and feeding inhibition of animals seems well established, it is possible, as noted earlier (8), to appraise preliminarily both the presence of this type of compound in natural sources and its potential bioactivity on the basis of its hot taste.

The inspection of the structure-activity relationship of bicyclic compounds, supporting the previous view (5,6) of interaction of the dialdehyde with primary amino groups in vivo, further suggests that the binding is possible only for compounds having a suitable distance between the two aldehyde groups.

Finally, although more data are needed on bulkier molecules, the inactivity of compounds possessing a tricyclic and tetracyclic skeleton would suggest that only smaller molecules, such as the bicyclic ones, fit the receptor sites.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined with a Kofler apparatus and are uncorrected. ^{1}H-nmr spectra were recorded on Bruker 500 and Bruker 250 instruments. Mass spectra were obtained on AEIMS-30 and MS-902 instruments. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Hplc was performed on a Waters 6000A apparatus using an R.I. detector Waters R401.

MATERIALS.—*Ent*-isocopal-12-en-15,16-dial [**8**] was isolated from *Spongia officinalis* (9). Scalaradial [**9**] was isolated from *Cacospongia mollior* (10). Warburganal [**3**] (11) and the *cis*-fused dialdehyde **6** (7) were synthesized as previously described. (±)-Isopolygodial [**2**] was prepared by Al_2O_3-catalyzed isomerization of (±)-polygodial (13).

SYNTHESIS OF (−)-POLYGODIAL [**1a**] AND (+)-POLYGODIAL [**1b**].—Ley's procedure (11) for the synthesis of racemic polygodial was employed to obtain the racemic diol mixture [**11** and **12**]. To a solution of the racemic diol (270 mg) in pyridine (5 ml) an excess of L(−)-menthoxyacetyl chloride (0.7 ml) was

[5]Compound **6** yields a pyrrole derivative analogous to the compound which is obtained from polygodial [**1a**] in the same conditions (5). A comparison between the tendency of these dialdehydes to react with methylamine in biomimetic conditions and their biological activity will be reported elsewhere.

added. The solution was kept at room temperature for 2 h and then poured into ice H_2O and extracted with Et_2O. The Et_2O solution was washed with H_2O and saturated $NaHCO_3$ and concentrated in vacuo. The crude product was subjected to hplc fractionation on a preparative μ-porasil column using *n*-hexane-EtOAc (97:3) as the eluent; 120 mg of **14** (less polar) and 169 mg of **13** were obtained using this procedure, along with 171 mg of mixed material. The recovered diastereomers **13** and **14** were 100% pure, as judged by hplc and ^1H nmr.

Compound **13**.—Ms *m/z* 416 (M^+-menthoxyacetic acid), 204; ^1H nmr δ ($CDCl_3$) 5.91 (1H, m), 4.60 and 4.57 (2H, ABq, J=12.2 Hz), 4.35 (1H, dd, J=11.6 and 3.3 Hz), 4.19 (1H, dd, J=11.6 and 6.9 Hz) 4.13 and 4.04 (2H, ABq, J=16.1 Hz), 4.08 and 4.01 (2H, ABq, J=16.2 Hz), 3.13 (2H, m), 0.92, 0.91, 0.90, 0.89, 0.89, 0.87, 0.81, 0.79, 0.78 (methyl signals).

Compound **14**.—Ms *m/z* 416 (M^+-menthoxyacetic acid), 204; ^1H nmr δ ($CDCl_3$) 5.91 (1H, m), 4.62 and 4.54 (2H, Bq, J=12.2 Hz), 4.38 (1H, dd, J=11.6 and 3.3 Hz), 4.14 (1H, dd, J=11.6 and 6.9 Hz), 4.14 and 4.04 (2H, Abq, J=16.1 Hz), 4.09 and 4.00 (2H, ABq, J=16.2 Hz), 3.14 (2H, m), 0.92, 0.91, 0.90, 0.89, 0.89, 0.87, 0.81, 0.80, 0.78 (methyl signals).

Compound **13** (169 mg) was dissolved in a 5% KOH-MeOH solution (10 ml) and stirred for 1 h at room temperature. The solution was concentrated in vacuo, diluted with H_2O, and extracted with Et_2O affording 66 mg of pure **11**, mp 69-71°; $[\alpha]^{25}D$ −6.9° (*c* 2.9, $CHCl_3$), having ms and ^1H-nmr spectra identical to those reported for the racemic compound (10). A solution of the diol **11** (59 mg) in anhydrous CH_2Cl_2 (2 ml) was added dropwise to a CH_2Cl_2 solution (2 ml) containing $(COCl)_2$ (0.1 ml) and anhydrous DMSO (0.16 ml) stirred at −60° under N_2 (Swern reagent). Stirring was continued for 30 min at −60°, and then triethylamine (0.2 ml) was added. The temperature was allowed to rise gradually to room temperature during 1 h, and the reaction mixture was poured into H_2O and extracted with CH_2Cl_2. Usual work-up gave 58 mg of crude product which was chromatographed on a Si gel column using petroleum ether-Et_2O (7:3) as the eluent, affording 34 mg of pure **1a**, $[\alpha]^{25}D$ −135° (*c* 2.0, $CHCl_3$), identical with an authentic sample (2).

Using a similar procedure, hydrolysis of compound **14** (120 mg) yielded 35 mg of diol **12**, mp 68-70°; $[\alpha]^{25}D$ −6.8° (*c* 1.4, $CHCl_3$), which on Swern oxidation, yielded 19 mg of **1b**, $[\alpha]^{25}D$ +128° (*c* 0.7, $CHCl_3$), whose ^1H-nmr spectrum was identical with that of **1a** and to that reported for (±)-polygodial (10).

Al_2O_3-CATALYZED ISOMERIZATION OF (±)-**6** TO (±)-**7**.[6]—A solution of (±)-**6** (18 mg) in Et_2O (4 ml) was absorbed on basic Al_2O_3 (4 g), and the mixture was stirred at room temperature for 30 min. Et_2O was added, and the mixture was filtered and evaporated in vacuo. The crude product (14 mg) was purified on semipreparative Si gel-tlc using petroleum ether-Et_2O (7:3) as eluent. Elution of the major uv absorbing band yielded 7 mg of (±)-**7** as an oil. Ms *m/z* 234 (M^+); ^1H nmr δ ($CDCl_3$) 9.86 (1H, d, J=2.5 Hz), 9.41 (1H, s), 7.10 (1H, m), 3.26 (1H, m), 0.96 (3H, s) 0.93 (3H, s), and 0.91 (3H, s).

HUMAN TASTE BIOASSAY.—Filter paper discs 1 cm in diameter were dipped into 0.5% Me_2CO solutions of the dialdehydes, air dried, and then tasted for hotness by a group of five people. Tasteless dialdehydes (Table 1) were further tested at 1% concentration giving the same result.

FEEDING INHIBITION BIOASSAY.—Laboratory bioassays were performed on two phytophagous insect species of great economic importance in agriculture: *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae; common name—Egyptian cotton leafworm) and *Leptinotarsa decemlineata* Say (Coleoptera: Crysomelidae; common name—Colorado potato beetle) both reared under laboratory conditions. Leaf discs, 25 mm in diameter, were cut out from suitable leaves and treated by dipping into Me_2CO-H_2O (10:90; containing 0.05% of Tween 20) solution of tested compounds.

Castor bean leaves were used for the *S. littoralis* test, potato leaves for the *L. decemlineata*. After drying, discs were placed on other discs of the same size and made of moistened polymeric paper Ferlosa ® (Mont edison), 1.5 mm in thickness and kept in Petri dishes. They were then infested with 10 third-instar larvae of the insect considered. Larvae were starved for ca. 3 h before introduction into the dishes.

For the "choice" method, two treated discs were compared with two other discs dipped into Me_2CO-H_2O (10:90; containing 0.05% Tween 20) in the same Petri dish, 10 cm in diameter. For the "no choice" method comparison was made between four treated discs and the same number of untreated discs in another Petri dish as control. The replicates were kept at 24±1° and 70±5% relative humidity, illuminated continuously by daylight-type fluorescent tubes. Each dose level and control had three replicates and reported values are the mean of at least five assays. After 24 h, leaf discs were removed and food consumption estimated as area loss by Portable Area Meter LI 3000 (LI-COR).

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[6](±)-**7** was previously prepared (7) by isomerization of (±)-**6** with 1,5 diazabicyclo[4.3.0]nonene-5 in an nmr tube, but the product was not isolated.

The antifeeding ratios were calculated according to the following formula:

$$\text{antifeeding ratio} = 1 - \frac{\text{area \% consumed in treated discs}}{\text{area \% consumed in untreated discs}} \times 100$$

ACKNOWLEDGMENTS

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