and the resulting antibody titers, obtained by Makinodan *et al.* in a system very similar to the present one (*14*). Also against this possibility are the results obtained in groups of mice that received a reduced number of untreated cells. Injecting only one-fourth (experiment 2), or one-fifth (experiment 1, Fig. 1), the number of cells as compared to the other groups, leads to an approximately equal reduction in both determination and competence titers. If there were compensation by proliferation on the part of the competent cells, only the determination titers should have been reduced.

The statistical significance of the actinomycin effect can be assessed simply. Within each experiment (that is, for each preparation of peritoneal fluid cells), the ratio of antibody titer due to determination as opposed to antibody titer due to competence, is smaller in every mouse that received actinomycin-treated (0.8 to 3.6 μM) cells, than in every mouse that received untreated cells. Since there are 27 mice in the former group, and 24 mice in the latter (including those which received reduced cell numbers) the probability for this result to occur by chance is $P << 0.0001$.

Two conclusions can be derived from the experiments presented.

1) The significantly higher sensitivity towards actinomycin D of antibody formation due to cellular determination establishes the immunologically determined cellular state as something different in character from the competent state.

2) Both determined cells and competent cells have to prepare for antibody formation (differentiate), and then produce antibody after the time when the actinomycin treatment takes place. But determined cells have had the necessary interaction with antigen before, and competent cells have it after the actinomycin treatment. It appears that whatever is retained in the cells from this interaction with antigen is more sensitive toward actinomycin than the capacity of these or other cells in the population to produce antibody in general. Since actinomycin interacts with DNA, and inhibits the transcription of information from DNA to RNA, this process appears to take part in determining the immunologically specific structure of antibody. For such a conclusion one possibility, however, has to be considered as a reservation. It is still conceivable that actinomycin, in these experiments, acts simply by destroying cellular functions in an immunologically nonspecific way. One would then have to assume, that by interacting with antigen and becoming determined, cells also change in physiological aspects—for example, their membrane permeability for actinomycin D. The differential effect might then just be due to a difference in the effective actinomycin concentration that reaches the chromosomes.

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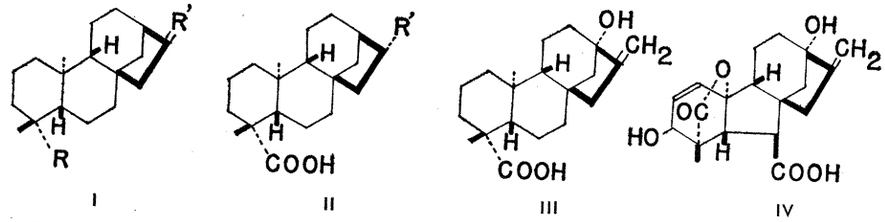
Growth Response of the d-5 and an-1 Mutants of Maize to Some Kaurene Derivatives

Abstract. *(—)-Kaur-16-en-19-oic acid and (—)-kaur-16-en-19-ol oxygenated derivatives of (—)-kaurene, stimulated seedling elongation for the two nonallelic dwarf mutants of maize,* d-5 *and* an-1. *Replacement of the exocyclic methylene group attached to ring D by a keto-, methyl-, hydroxymethyl-, carboxy-, or methylcarboxy group resulted in compounds which were biologically inactive. These kaurene derivatives are structurally related to the gibberellins which produce a similar type of elongation for the* d-5 *and* an-1 *mutants.*

Several neutral and acidic diterpenoid metabolites have been isolated and characterized from the flowering plant, *Ricinocarpus stylosus* Diels; in addition, a number of derivatives of these compounds have been prepared (*1*). The possible metabolic relationship of some of these compounds to the gibberellins and to the kaurene derivative, steviol, has led us to test their biological activity as gibberellin-like substances, that is, their stimulation of organ elongation when applied to seedlings of certain flowering plants (*2*).

Thus far eight new compounds (Fig. 1, compounds 2–9) have been tested



I

1. R : CH_3, R′ : CH_2 (Kaurene)
2. R : CH_2OH, R′ : CH_2 (Kaurenol)
3. R : COOH, R′ : CH_2 (Kaurenoic acid)
4. R : CH_2OH, R′ : O
5. R : COOH, R′ : O

II

6. R′ : CH_3
7. R′ : CH_2OH
8. R′ : COOH
9. R′ : $COOCH_3$

III

10. (Steviol)

IV

11. (Gibberellic acid) (GA-3)

Fig. 1. Structure of compounds tested for biological activity. Gibberellic acid (IV-11), steviol (III-10), the kaurenoic acid (I-3), and the kaurenol (I-2) were active on *d-5* and *an-1* mutants of maize. The kaurene derivatives, (I-4), (I-5), (II-6), (II-7), (II-8), and (II-9), were biologically inactive.

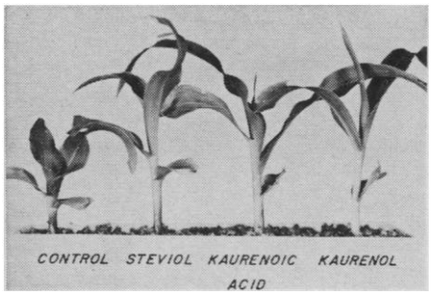


Fig. 2. Growth response of *d-5* mutants of maize to 100 μg of kaurenol, kaurenoic acid, and steviol. Seedlings were treated at the time of emergence of the first leaf from the coleoptile.

for biological activity and compared with gibberellic acid (GA-3) (*3*) and steviol. Two of these eight compounds, a kaurenoic acid (*4*) and a kaurenol, have been found to stimulate leaf sheath elongation for the *d-5* and *an-1* dwarf mutants of *Zea mays L.* Steviol, reported to be biologically active for the *d-5* mutant (*5*), was also active for the *an-1* mutant. Kaurene has been reported to be inactive as a gibberellin-like substance (*6*), although it is incorporated into GA-3 by the fungus *Fusarium moniliforme* Sheldon (see *7*).

All of the kaurene derivatives tested (Fig. 1, compounds 2–10) were found to be inactive in other gibberellin-elongation assays—tests on *d-1* and *d-2* mutants of maize (*2*), the rice seedling (*8*), the cucumber hypocotyl (*9*), the dwarf pea (*10*), and the morning glory seedling (*11*). Each compound was routinely tested in doses of 50 μg or 100 μg. All compounds were applied in aqueous solution or in 95 percent ethanol.

At a dosage of 100 μg, the amount of seedling elongation for the *d-5* mutant was approximately the same for the kaurenoic acid, the kaurenol, and steviol (Fig 2). The lengths of leaf sheaths of treated mutants were on the average 3.5 cm greater than those of non-treated dwarf controls. In this test, a response 1.2 cm over the mean shown by leaves of dwarf controls was significant at the 5-percent level. When the responses to 50-μg doses were compared to a standard curve of response to GA-3, the kaurenoic acid and the kaurenol were approximately 1 percent as active as GA-3 for the *d-5* mutant. Steviol has also been reported to be appreciably less active than GA-3 for the *d-5* mutant (*4, 5*). The type of growth response associated with the kaurenoic acid and the kaurenol was visually indistinguishable from a gibberellin response; 10-μg dosages applied at daily intervals over a period of 10 days resulted in *d-5* seedlings which appeared very similar to nontreated normals.

Comparison of the three biologically active kaurene derivatives with kaurene suggests that neither oxygenation of the C-19 position to the acid, nor hydroxylation at the C-13 position (as in steviol) is necessary for biological activity in this system (*d-5* and *an-1* mutants). It would also appear that the exocyclic ethylenic bond of the kaurene ring system is necessary for biological activity in the *d-5* and *an-1* assays, since the norketo derivatives of the kaurenoic acid and the kaurenol (I-4 and I-5) were inactive, as were the kauranoic acid (II-6) and the C-17-oxygenated derivatives of the kauranoic acid (II-7, II-8, and II-9).

The biological activities of the kaurene derivatives and the gibberellins may reflect similarities in active sites. However, it is also possible that these compounds are metabolically related, such that the kaurenoic acid, the kaurenol, and steviol are precursors that can be converted to gibberellins by the *d-5* and *an-1* mutants of maize.

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Intestinal Disaccharidases: Absence in Two Species of Sea Lions

Abstract. *Pups of both the California and Stellar sea lions have no intestinal enzymes for hydrolysis of sucrose, lactose, cellobiose, or trehalose. If these animals were given lactose or sucrose, they developed severe fermentative diarrhea and weight loss similar to the clinical syndrome encountered in infants with either hereditary or acquired intolerance to disaccharides.*

In 1962 Pilson and Kelley (*1*) reported that lactose was absent from the breast milk of the California sea lion (*Zalophus californianus*). This milk contained no detectable carbohydrate (*2*). Because of the possible role for substrate as an inducer of enzymes during development, it was of some interest to ascertain whether lactase or any other disaccharidase was present in the intestine of these animals and whether the sea lion could digest various disaccharides.

Sea lion pups (6 to 8 weeks old) were captured on the Coronado Islands, Mexico, and were housed at the hospital of the San Diego Zoo in a cage containing a pool of fresh water (*3*). Although feeding with nipple and bottle has been successful for pups of the Stellar sea lion (*Eumetopias jubata*) (*4*) and of the walrus (*Odobenus divergens*) (*5*), our pups of the California sea lion were force fed through a gastric tube which was passed through a hole in a rubber mouth gag, a method similar to that employed in feeding the sand seal (*Phoca vitulina*) (*6*). Initially, a mixture containing 10 percent casein and 32 to 35 percent cod liver oil was used for the diet. However, since casein contains approximately 0.75 percent lactose, boiled eggs were substituted to supply the needed protein; vitamins B and C were added daily. In more recent studies a diet containing 10 percent meat-base protein (*7*) and 35 percent corn oil has been used.

The animals were tested for their capacity to digest and absorb carbohydrates. Either glucose, lactose, or sucrose (1.75 to 2.0 g per kilogram of body weight) was administered by gastric tube as a 15 percent solution. Initially, blood was obtained from the brachial vein just lateral to the axilla, but in later studies samples were taken by cardiac puncture. Glucose was measured by the glucose oxidase method on a Somogyi filtrate of blood (*8*).