flat cells, after more than 200 days in culture, continue to secrete ACTH-like material into the medium; however, the log dose response to flat-cell culture medium is no longer parallel to the response obtained with porcine ACTH. The meaning of this change is not yet known. The material secreted by cells in culture more than 200 days may be a different compound from that secreted by the early cultures; it may not be free, native ACTH; it may not be an ACTH-like peptide at all, but another substance that stimulates steroidogenesis by adrenocortical tumor cells.

Clonal, testicular Leydig-cell cultures (Fig. 1d) secreted Δ^4-3-keto-steroids into the medium at a rate of 1.0 μg/mg of protein per hour after 12 months in continuous culture. Dark brown pigment was formed by clonal melanoma cells maintained in culture for over a year (Fig. 1e).

Our experiments show that serially-propagated clonal strains of animal cells can perform specialized, organ-specific functions for prolonged periods. The marked stability of functional properties after cloning implicates selective overgrowth as a major factor in the loss of specialized function in culture. Nearly limitless numbers of isolated functional cells for physiological, biochemical, and embryological experiments can thus be obtained by the combination of alternate animal and culture passage and cloning techniques.

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12. Supported in part by research grants from NIH (CA-04123 and AM-11011) and NSF (GB-1641) and by a NIH career development award to A.H.T. Publication No. 434 from the Graduate Department of Biochemistry, Brandeis University. We thank J. Thivierge for technical assistance.

4 October 1966

Germination of Witchweed (Striga lutea Lour.): Isolation and Properties of a Potent Stimulant

Abstract. *A crystalline germination stimulant (trivial name strigol) for the root parasite, witchweed* (Striga lutea *Lour.), has been isolated from cotton root exudates and characterized as a $C_{19}H_{22}O_6$ compound. Although apparently different from known plant hormones, the stimulant is active at hormonal levels, causing germination at concentrations less than 10^{-5} part per million.*

Witchweed (*Striga lutea* Lour.) (*1*) is an angiospermous root parasite indigenous to several tropical and subtropical areas of the Eastern Hemisphere. The discovery of *S. lutea* in the coastal plain section of the Carolinas in 1956 has necessitated a costly quarantine and control program (*2*), and its depredations in the food-poor countries of the Eastern Hemisphere have long been a source of concern. With few exceptions, seeds of the parasite [and related parasites such as *Striga hermonthica* (Del.) Benth] remain dormant until germination is stimulated by a chemical produced by the host plant or by certain other plant species (*3*). Identification of the stimulant and an understanding of the germination process seem basic to a rational control program. In addition, this information could provide an opening into fundamental knowledge regarding seed germination in general. The identity of the stimulant (or stimulants) has been the object of considerable research (*4*), but the low concentrations in which it is produced and its relative instability have hitherto prevented isolation in pure form.

We wish to report the isolation of a crystalline, highly active germination stimulant for *S. lutea* from cotton root exudates. Cotton plants were grown hydroponically and the nutrient fluid periodically circulated through charcoal columns. The charcoal saturated with stimulant was eluted with acetone, and the resulting aqueous acetone solution was evaporated at reduced pressure to leave an aqueous solution of the stimulant (*4*). Extraction with benzene at this point gave a 30-fold concentration of active material in the organic layer. The residue from evaporation of this layer was separated by preparative thin-layer chromatography on silica gel into two stimulatory substances, *A* and *B*, which were purified by further chromatography. Crystallization of *A* from acetone-hexane or benzene-hexane gave pure *A* as white needles. We propose the trivial name strigol for this compound.

The stimulant activity of purified materials was compared by determining their effect on the germination of *S. lutea* seeds pretreated with water (*5*). Crystalline strigol produced 50 percent germination at concentrations of less than 10^{-5} part per million in water, and some noncrystalline preparations of stimulant *B* were at least as active.

The melting point of strigol varies somewhat with the rate of heating, but the purest sample melted at 200° to 202°C with decomposition after some softening around 195°C (Kofler apparatus). It is readily soluble in moderately polar solvents (for example, acetone, methylene chloride), moderately soluble in benzene, and relatively insoluble in hexane.

The infrared spectrum of strigol in methylene chloride (Fig. 1) has bands at 3590, 1787, 1745, 1682, and 1601 cm^{-1}; the 1787 band is the most intense. In samples crude enough to be soluble in carbon disulfide, the two high-frequency carbonyl bands appear at 1795 (more intense) and 1755 cm^{-1}, whereas in chloroform they shift to 1786 and 1740 cm^{-1}, and the 1740 cm^{-1} band is the more intense. There is an ultraviolet maximum at 236 mμ (molar extinction coefficient about 18,000). Prominent features indicated by nuclear magnetic resonance spectra (*6*) are two methyl groups (at 1.16 and 1.08 δ from internal tetramethylsilane), a methyl or methylene group attached to an unsaturated system (broadened singlet at 2.00 δ), and a highly deshielded hydrogen (7.42 δ).

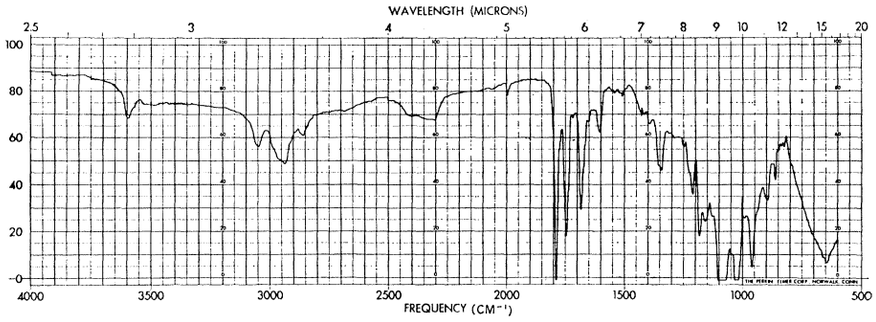

Fig. 1. Infrared spectrum of strigol in methylene chloride solution at a concentration of 2.2 mg/ml; cell thickness of 0.2 mm; 5× scale expansion.

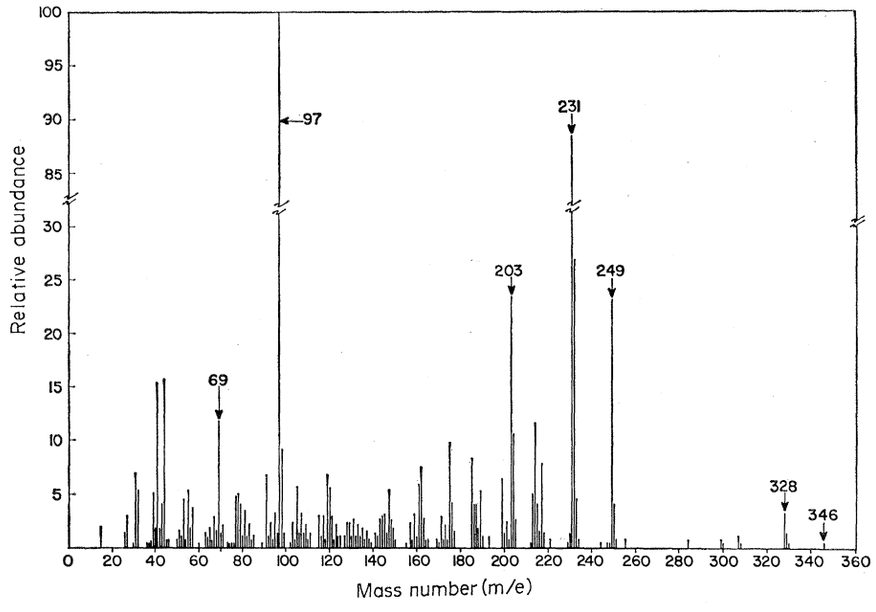

Fig. 2. Mass spectrum of strigol.

The mass spectrum (*7*) (Fig. 2) has the M+ peak at *m/e* 346. Due to its low intensity, a high-resolution measurement could not be made on this ion. However, the peaks at *m/e* 249, 231, and 97 were measured by peak matching at high resolution. Values of 249.1132 (calculated for $C_{14}H_{17}O_4$, 249.1127), 231.1017 (calculated for $C_{14}H_{15}O_3$, 231.1021) and 97.0307 (*8*) (calculated for $C_5H_5O_2$, 97.0290) were obtained.

On the assumption that the 249 and 97 ions result from a single fragmentation pathway, addition gives a molecular formula of $C_{19}H_{22}O_6$ (*9*). The major fragmentation patterns appear to be

$$346 \xrightarrow{-18} 328 \xrightarrow{-97} 231 \xrightarrow{-28} 203;$$

$$346 \xrightarrow{-97} 249 \xrightarrow{-18} 231;$$

$$346 \xrightarrow{-249} 97 \xrightarrow{-28} 69.$$

In support of this, metastable ions were observed at *m/e* 49.1 (97 → 69), 178 to 179 (very broad, 231 → 203 and 346 → 249), and 214.3 (249 → 231).

The presence of a hydroxyl group in strigol (compare infrared) has been confirmed by acylation. The mild conditions necessary for this reaction show that the hydroxyl is not tertiary, and manganese dioxide oxidation of noncrystalline material suggests that it is allylic (formation of α,β-unsaturated ketone).

The infrared spectrum of stimulant *B* differs from that of strigol mainly in the presence of bands attributable to the *O*-acetyl group (1741 and 1238 cm^{-1} in carbon disulfide). The acetyl derivative of strigol was chromatographically indistinguishable from *B* and the infrared spectra were quite similar. Considering the fact that these data were obtained on noncrystalline materials, the evidence suggests that *B* is strigyl acetate.

Although other assignments are possible, the position of the high-frequency carbonyl bands and their change in intensity and position in various solvents suggest the presence of a $\Delta^{\alpha,\beta}$-butenolide moiety (*10*). (In this connection it is of interest that the cardiac glycoside sarveroside, which contains a butenolide group, causes 50 percent germination at 30 parts per million.)

The molecular formula assigned to strigol is the same as that for gibberellic acid, which has a function in seed germination (*11*). However, strigol is apparently not identical with any of the known gibberellins, and gibberellic acid does not stimulate witchweed seed germination (*12*).

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8. Lack of sufficient material made this latter result somewhat less reliable than the other two values.
9. The presence of sulfur could be ruled out by the high-resolution mass spectrum, as could the presence of nitrogen. Furthermore, combustion analysis of stimulant *B* showed less than 0.1 percent nitrogen. The carbon and hydrogen percentages were in reasonable agreement with the formula $C_{21}H_{24}O_7$ for *B*, as would be expected if *B* is strigyl acetate. (Analysis by Microtech Laboratories, Skokie, Illinois.)
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26 August 1966