the maximum possible radiation dose due to solar α-particles.

These represent the doses experienced on the surface of a man who is inside a spacecraft of designated thickness. We believe the fluxes on which these doses are based are correct to within a factor of 2 for shielding of 1 g/cm² for the first five events in Table 1. For the other events at 1 g/cm² and for all events at the other thickness this flux is believed to be accurate to ± 25 percent.

In the absence of any shielding considerations we shall assume that the typical spacecraft or satellite-observing station will have a wall thickness approaching 2 g/cm². Under these conditions a number of events over the last solar cycle would have provided integrated particle doses in excess of 200 rads. During the period from 10 to 20 July 1959 the total dose was 490 rads and from 12 to 18 November 1960 the total was 488 rads. The flares on 23 February 1956, 26 March 1958, 7 July 1958, and 10 May 1959 all produced doses greater than 100 rads inside a spacecraft with shielding of 2 g/cm².

The assessment of the damage caused by these doses is a difficult and, as yet, unsolved problem. First of all, the damage depends upon the exposure of certain critical areas of the body such as the eyes, brain, liver, and so forth, to the radiation. It may be possible for the pilots to assume positions or take up locations which would greatly reduce the dose to critical areas. Secondly, it is very difficult to evaluate the effectiveness of the heavily ionizing particles, especially in the killing of individual cells.

It seems reasonable to assume, however, that any event producing more than 100 rads in a period of 1 week is capable of causing enough damage at least to reduce the efficiency of a man appreciably if no extra precautions are taken. The larger doses may render him completely inoperative and cause failure of the mission. An increase of wall thickness in spacecraft to 5 g/cm² (10 lb/ft²) would reduce the dose in the most extreme event to less than 135 rads.

The extent of the hazard of these solar cosmic-ray outbursts depends essentially on encountering one of the large events. As we have seen, there are six to eight events which occurred during the last solar cycle that could possibly present a serious problem in space in terms of immediate damage to a man. The cumulative effects of smaller events considerably exceeds accepted tolerance doses, but should not result in the failure of a specific mission. Of the major solar events, one occurred in 1956, two in 1958, two in 1959, and one in 1960; that is within 3 years of the peak of the sunspot cycle occurring in 1958. Evidence from periods near the last two sunspot minimums and the one we are entering now indicate that there are periods of 3 to 4 years when no large events would be expected to occur.

For short missions of a few days or weeks, such as trips to the moon or for orbiting stations in the vicinity of the earth, we may attempt to predict the possibility of a major event by studying local conditions on the sun. If "solar weather predictions" are used it should be possible to travel at periods of sunspot maximum. At a sacrifice of approximately 50 percent of the available time, it should be possible to decrease the chance of encounter with a large event by a factor of about 10 for a predicting period of 2 weeks. This reduces to about 1 percent the probability of encounter with a large flare event for a two-week voyage.

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Abscisin II, an Abscission-Accelerating Substance from Young Cotton Fruit

Abstract. *Crystalline abscisin II, with a tentative molecular formula of* $C_{15}H_{20}O_4$, *has been isolated from young cotton fruit. It accelerates abscission when applied in amounts as low as 0.01 μg per abscission zone. It inhibits indoleacetic acid-induced straight growth of* Avena *coleoptiles but has no gibberellin activity on dwarf maize.*

Endogenous abscission-accelerating substances are now known to occur widely in the higher plants (*1*). Recently, abscisin (I) was isolated from the mature fruit wall of cotton (*2*), but little is yet known of its physiological properties. The abscission-accelerating substance which is best known physiologically occurs in the young fruit of cotton; its activity reaches a peak at the time of the onset of young fruit abscission (*1*). We now report the isolation of this substance, here named abscisin II, and describe some of its chemical and physiological properties.

Abscission-accelerating activity was measured with explants (excised cotyledonary nodes) of 14-day-old cotton seedlings. Seedlings were grown at 32° ± 2°C with a 15-hour photoperiod of 22,000 lu/m² provided by "warm-white" fluorescent lamps. The explants consisted of 3-mm stumps of the cotyledonary petioles and of the stem, and a 10-mm stump of the hypocotyl. Explants were placed upright in stainless steel holders in petri dishes containing a 5-mm layer of 1.5-percent agar. Fractions to be tested were applied to the petiole stumps in 5-μl droplets of 1.0-percent agar. Dishes with explants were kept in the dark at 30°C. Abscission was determined by applying a force of 5 g to the end of the petiole stumps at daily or more frequent intervals.

Four- to seven-day-old fruit was quick-frozen in the field with dry ice, stored at −5°, and later lyophilized to approximately 10 percent moisture. A 78-kg sample (225 kg fresh weight) was then extracted overnight at ambient temperature of 20° to 25°C with 520 liters of 80-percent acetone. After filtering and concentrating, the remaining liquid was adjusted to *p*H 2.0 with dilute HCl and extracted twice with equal volumes of ethyl acetate. The ethyl acetate phase was extracted three times with 2.0 percent aqueous sodium bicarbonate. The

sodium bicarbonate phase was acidified to *p*H 2.0 and extracted twice with equal volumes of ethyl acetate. The remaining acid fraction, weighing 147 g, was separated by adsorption chromatography. Carbon (Darco G-60) and celite (Hyflo Super-cel) (1 : 2 by weight) were thoroughly mixed in water and packed in a column by stirring. The acid fraction was applied in a ratio of approximately 1 : 10 (acids to carbon) and eluted with increasing concentrations of acetone in water. Each of the ten fractions (10 to 100 percent) contained 3 liters of solvent per 100 g carbon. A total of 4.15 g oily material having abscission accelerating activity was found in the 50-percent and 60-percent acetone eluates. This was applied to a silicic acid–celite (1 : 2 by weight) column packed in chloroform, in a ratio of 1 g sample per 20 g silicic acid. Fractions were eluted successively with increasing concentrations of ethyl acetate in chloroform (one liter of solvent per 50 g silicic acid) starting with 5 percent ethyl acetate. Peaks of abscission-accelerating activity were found in the 10- to 30-percent ethyl acetate, and in the 50- to 60-percent ethyl acetate eluates. Only those fractions obtained with 10- to 30-percent ethyl acetate were purified further. Eluates were combined and evaporated to dryness under reduced pressure. The residue was treated with a small amount of chloroform from which an insoluble crystalline material, having no abscission activity, was eliminated by filtration. The remaining 238 mg of chloroform-soluble material was streaked on four sheets of Whatman 3 mm chromatographic paper (56.3 × 45.6 cm) and developed to 35.0 cm with iso-propanol : ammonia : water (10 : 1 : 1 vol/vol).

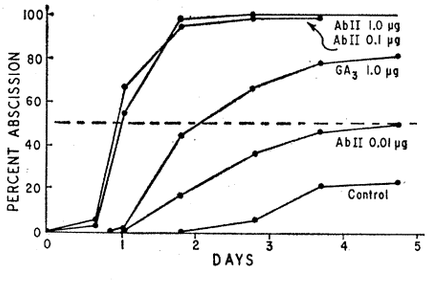

Fig. 2. Abscission acceleration induced by applications of abscisin II to petiole stumps of explants of cotton seedlings. Each treatment included 30 explants (60 abscission zones). For comparison, the effect of applying an optimum concentration of gibberellin A_3 is also shown.

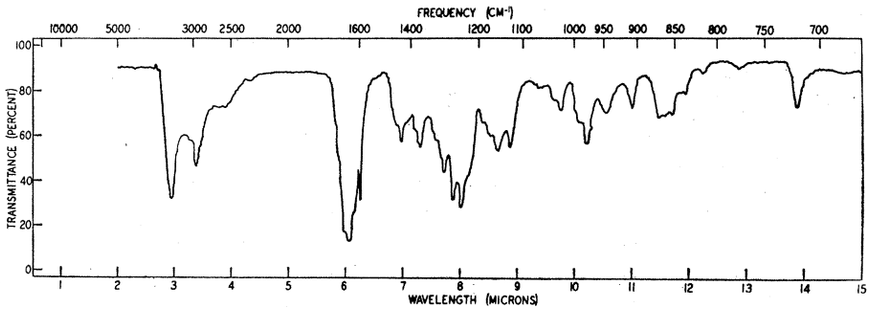

Fig. 1. Infrared spectrum of abscisin II.

Each paper was divided into 10 portions according to R_F and eluted with methanol by macerating in a Waring blendor. Eluates were evaporated to dryness, dissolved in water at *p*H 7.0, and filtered. The filtrates were acidified to *p*H 2.0, extracted twice with ethyl acetate, and evaporated to dryness. Eluates from R_F 0.4 to 0.8 were combined (66 mg) and applied to a silicic acid–celite column as described above. The 10-percent ethyl acetate eluate was concentrated to 23 mg of semisolid oily material at reduced pressure. After recrystallizing twice from chloroform-petroleum ether, 9 mg of highly active crystals of abscisin II were obtained.

The purity of the crystals was tested by thin-layer and paper chromatography. Nine different solvent systems were used; in each instance only one spot was detected with potassium permanganate spray.

Abscisin II has a melting point of 160° to 161°C and sublimes at 120°C. It is an acidic, colorless compound, soluble in aqueous sodium bicarbonate, chloroform, acetone, ethyl acetate, diethyl ether, methanol, and ethanol; slightly soluble in benzene and in water; and sparingly soluble in petroleum ether. Its ultraviolet absorption maximum in methanol is 252 mμ (ε 25,200); its infrared absorption spectrum in KBr pellets is shown in Fig. 1. The molecular weight of abscisin II is 264 (determined from mass spectrometry) and it contains 68.76 percent carbon and 7.96 percent hydrogen. Tests for nitrogen, sulfur, and halogens were negative. Therefore, $C_{15}H_{20}O_4$ was assigned to abscisin II as a tentative molecular formula. These data show that abscisin II is chemically distinct from abscisin I, isolated from mature fruit walls of cotton by Liu and Carns (*2*).

Abscisin II is a very effective abscission accelerator. Figure 2 shows the results of applying three different amounts of abscisin II to the petiole stumps of explants. Acceleration resulting from the application of 1.0 and 0.1 μg is greater than can be obtained from maximum accelerating amounts of gibberellin A_3. Evidence indicating that abscisin II is an abscission-accelerating plant hormone is presented elsewhere (*1*).

Abscisin II was also tested for its growth-inhibiting and gibberellin activities. In the presence of 0.1 μg/ml indoleacetic acid, it completely inhibited *Avena* coleoptile straight growth at concentrations of 3.0, 10.0, and 30.0 μg/ml; at 0.1 μg/ml it reduced growth to 60 percent of that induced by indoleacetic acid alone. No gibberellin activity was found when applications of 0.25 to 50.0 μg/per plant were made to dwarf maize mutants d_1, d_3, and d_5 (*3*).

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3. These investigations were supported in part by funds from the National Cotton Council administered through the U.S. Department of Agriculture and a contract with the U.S. Army Biological Laboratories. Cotton fruit for extraction was obtained with the cooperation of the personnel at the U.S. Cotton Field Station, Shafter, California. We thank Margaret DeCasper and Nancy Beck for their assistance; Paul Allen of the Department of Agricultural Toxicology, University of California, Davis, for determinations of infrared spectrums and ultraviolet absorption; Department of Chemistry, Stanford University for mass spectrometry determinations and elemental analysis; and Dr. R. Cleland for providing the facilities for the *Avena* straight-growth tests.
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