tated by heavy metal ions (namely, Hg, Pb, Ag, Ba).

The melting point, nitrogen content, and degree of toxicity of the crystals obtained by the latter method indicate that these crystals are identical to those isolated by Schilling (*1*) who reported: mp 193° to 194°C (dec.); nitrogen, 21.1 percent. It would appear that the crystals isolated in this laboratory represent a somewhat higher degree of purity than those reported by Schilling.

The methods of isolation reported in this paper are not suited for the large-scale preparation of the toxic factor from sweet peas, since the yields in terms of percentage of original toxicity are very low. Because of the limited solubility of the toxic factor in alcohol, it is impractical to attempt to extract *completely* the factor from sweet peas with this solvent. However, the extraction with alcohol yields a toxic extract that contains less unwanted material than do the aqueous solvents used by other investigators for extracting *Lathyrus* seeds (*1, 5–7*). The crystallization of the toxic substance directly from the alcohol extract is dependent upon the prior removal of the lipids from the sweet peas. Thus, when the extraction with the Skelly Solve B was inadequate, either no crystals subsequently formed in the alcohol extract, or a difficultly manageable mixture of resin and crystals was obtained.

Addendum. Dupuy and Lee have recently reported the isolation of an active crystalline material from *Lathyrus pusillus* (*8*). Schilling's method for the isolation of toxic crystals from sweet peas (*1*) was apparently similar to the procedure used by Dupuy and Lee.

References and Notes

1. E. D. Schilling, *Federation Proc.* **13**, 290 (1954).
2. I am grateful to Raymond H. Coulter of the Ferry-Morse Seed Co., Detroit, who generously supplied the sweet peas used in these studies.
3. Amberlite IR-105 is no longer available. It seem to have been replaced by Amberlites IR-112 and IR-120. Neither of the latter has been tried in this procedure.
4. Nitrogen determinations were made by Elizabeth Huang.
5. A. R. Schubert and H. B. Lewis, *Proc. Soc. Exptl. Biol. Med.* **31**, 86 (1952).
6. H. B. Lewis *et al., J. Nutrition* **86**, 537 (1948).
7. B. J. Geiger, H. Steenbock, and H. T. Parsons, *ibid.* **6**, 427 (1933).
8. H. P. Dupuy and J. G. Lee, *J. Am. Pharm. Assoc., Sci. Ed.* **43**, 61 (1954).
9. *Note added in proof*: Since this paper was submitted for publication, Schilling and Strong (*10*) have reported that the compound that was isolated by them (*1*) was β-(γ-L-glutamyl)-aminopropionitrile. The material isolated by the second method given in this paper has proved, through an exchange of samples with Strong (*11*), to be identical to that of Schilling and Strong. However, the toxic crystals that were isolated following treatment with ion-exchange resins (first method described in this paper) could not have been the glutamyl aminopropionitrile, since they were in the form of an essentially neutral sulfate of an amine. It is considered likely that these crystals were the sulfate of β-aminopropionitrile. If this is true, the toxic properties of the sweet pea factor must lie in the aminopropionitrile portion of the molecule. Unfortunately, all the available sulfate was required for toxicity studies. More is in preparation.
10. E. D. Schilling and F. M. Strong, *J. Am. Chem. Soc.* **76**, 2848 (1954).
11. F. M. Strong, personal communication.

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Exhaustive Chlorination of Hinokitiol (4-Isopropyltropolone)

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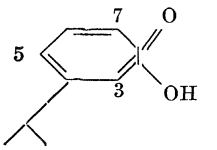
Hinokitiol, isolated from the phenolic part of the essential oil of *Chamaecyparis obtusa* Zieb et Zucc., is 4-isopropyltropolone (*1*). From this structure, it is



seen that it undergoes electrophilic substitution reactions preferably at 3-, 5-, and 7-positions (*2, 3*). Thus when it is chlorinated, for example, there will be three isomerides each of mono- and di-, and one of trisubstituted products. Nozoe, Sebe and associates obtained three monochlorohinokitiols (5-, mp 118.5° to 119.5°C; 7-, mp 47.5° to 48.5°C; 3-, mp 46.5° to 47.5°C (*4*) and three dichlorohinokitiols [3,5-, mp 81° to 82°C (*4*); 3,7-, mp 125° to 126°C (*5, 6*); 5,7-, mp 103° to 105°C (*5*)]. But trichlorohinokitiol has not been reported.

The exhaustive chlorination of hinokitiol gave the trichloro-compound, being isolated as sodium salt.

Through two wash bottles containing concentrated sulfuric acid and a flowmeter, chlorine gas was introduced at a constant velocity of 170 ml/min into a solution of hinokitiol (30.8 g) in glacial acetic acid (200 g) under mechanical stirring, until 5 mole equivalents of chlorine had been absorbed. The reaction mixture was worked up, a large amount of acidic oily products was removed, and the trichloro-compound was obtained from the phenolic portion in the form of sodium salt. After recrystalization from ethanol, the sodium salt melted at 232° to 233°C (decomposition). Analysis for $C_{10}H_8O_2Cl_3Na$—calculated: C, 41.48; H, 2.78; Cl, 36.74; found: C, 41.22; H, 2.88; Cl, 36.96.

Chlorination in an acetic acid (200 g) solution of hinokitiol (30.8 g) at 7°C over a period of 5 hr produced saturation.

Subtracting 1.2 mole equivalents (17 g) that were absorbed by the solvent under the experimental conditions, 5 mole equivalents reacted with hinokitiol.

Nozoe *et al.* (*1*) reported that it is difficult to obtain monochloro-compound when hinokitiol is chlorinated with only 1 mole equivalent of chlorine, the products being always contaminated by some dichloro-compounds, whereas monobromo-compound is obtained in a good yield. Chlorination in an acetic-acid (200 g) solution of hinokitiol (30.8 g) at 7°C during a period of 5 hr produced saturation. When the reaction mixture saturated with 6.0 mole equivalents of chlorine was allowed to stand at room temperature without a stopper, a loss of about 4 mole equivalents of chlorine was noted, hydrogen chloride being liberated.

We wish to thank T. Nozoe and E. Sebe for their kind advice.

References and Notes

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3. Y. Kurita, T. Nozoe, and M. Kubo, *J. Chem. Soc. Japan* **71**, 543 (1950): *Bull. Chem. Soc. Japan* **24**, 10 (1951); *Nature* **167**, 688 (1951).
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Lysis of Cultures Devoid of Vi Antigen by Vi I Bacteriophage of *Salmonella typhosa*

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The concept that the sites for bacterial receptors involved in antigen-antibody reactions correspond to those that determine phage host interactions was developed by Hadley (*1*) and by Burnet (*2, 3*) for certain *Salmonella* serotypes. Levine and Frisch (*4*) observed that differences in the somatic antigens of *Salmonella* group C could be detected by characteristic phage adsorption behavior. Although there are many examples of striking correlations between phage susceptibility and the distribution of heat-stable agglutinogens in various bacterial groups, it soon was observed that there also were many exceptions. This led Burnet (*3*) to explain these discrepancies in terms of the existence of an intimate relationship between the phage and the antibody surface rather than by the assumption of identical adsorption sites for phage and for antibody.

Craigie and Brandon (*5*) described the isolation of a bacteriophage that was specific for the heat-labile Vi antigen of the typhoid organism. The specificity of certain phages for the Vi form of *Salmonella typhosa* led to the demonstration of phage types of this organism by means of preparations of type II Vi phage (*6*). Several Vi phages have been described that are serologically distinct and differ also in respect to particle size, thermal death point, and lytic activity for Vi forms of *S. typhosa*. They have been tested on a great variety of salmonellae, shigellae, *Escherichia coli*, and on Vi and W forms of many strains of *S. typhosa* (*6, 7*). So far as we are aware, none of the Vi phages has been shown to lyse any culture that does not contain Vi antigen, although they are known to attack cultures other than *S. typhosa*. Nicolle *et al.* (*8*) reported that Vi phages lysed *Salmonella paratyphi C* (East Africa), certain cultures of the Bethesda-Ballerup group of paracolons, and cultures described by Kauffmann as *E. coli* (2624-36 and 5396-38).

The object of this report is to present evidence that the specificity of Vi phage I (*6*) is not dependent on the presence of a bacterial component that can be recognized serologically as Vi antigen. This study was initiated as the result of a chance observation of the lysis by typhoid Vi I phage of a culture of *Escherichia freundii* that did not contain Vi antigen. Examination of additional cultures of this species and of their slow lactose-fermenting analogs (*9*) (Bethesda-Ballerup paracolons) revealed that this was not a rare occurrence. Table 1 shows the relationship that exists in these cultures between susceptibility to typhoid Vi I phage and the presence of Vi antigen. The cultures that contained Vi antigen but were unaffected by the phage must be subjected to a more critical examination before it can be stated unequivocally that they are resistant.

The nature of the host-virus relationship in these cultures that appeared to lack Vi antigen was studied along the following lines: (i) comparison of the specificity and serologic nature of Vi I phage propagated on *E. freundii* and on phage type F_1 of *S. typhosa*, and (ii) demonstration of the absence of Vi antigen in certain cultures of *E. freundii* and paracolon bacteria that were lysed by the typhoid Vi I phage. The former was accomplished by the propagation of typhoid Vi I phage on a Vi negative culture of *E. freundii* through four single plaque passages. The starting material was a preparation of Vi I phage that had been propagated on phage type F_1 of *S. typhosa*. This material had been prepared in the Central Enteric Reference Laboratory under the direction of A. Felix and was dated July 1948. The stock of Vi I phage that had been propagated on *E. freundii* 10 was tested in parallel with the Vi I phage received from England by applying both of these to each of the 32 recognized phage types of *S. typhosa*. The results given by the two phage preparations were identical. Even the weaker lysis of types B3, D4, and M given

Table 1. The relationship between susceptibility to Vi bacteriophage and the presence of Vi antigen in *E. freundii* and related paracolons.

Species	No. of cultures	Action of Vi I phage	Vi antigen	Minimum No.* of O groups represented
E. freundii	9	+	−	4
	5	−	+	5
	16	−	−	9
Bethesda-Ballerup paracolons	5	+	−	3
	2	+	+	1
	6	−	+	2
	41	−	−	28

* Some *E. freundii* cultures possessed O antigens that could not be recognized with the serums available.