References and Notes

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1. L. C. Craig *et al.*, *Anal. Chem.* **23**, 1236 (1951).
2. L. C. Craig and D. Craig, in *Technique of Organic Chemistry*, Weissberger, Ed. (New York, Interscience, 1950), vol. III, p. 295.
3. We are indebted to Proctor & Gamble Co. for partial support of this study.
4. D. E. Bland, W. E. Hillis, and E. J. Williams, *Australian J. Sci. Research*, Ser. A **5**, 346 (1952).
5. C. R. Lancaster, E. B. Lancaster, and H. J. Dutton, *Am. Oil Chemists' Soc.* **27**, 386 (1950).
6. P. L. Nichols, Jr., *Anal. Chem.* **22**, 915 (1950).

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Isolation of Toxic Crystals from Sweet Peas (*Lathyrus odoratus*)

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Schilling (*1*) has reported the isolation from sweet peas (*Lathyrus odoratus*) of crystals that are active in the production of skeletal changes in rats. Active crystals that appear to be identical to these have been isolated also in this laboratory (*2*). The procedure most recently used in this laboratory is simple and seems to give crystals of good purity.

The first active crystals that I prepared were obtained with the aid of ion-exchange resins. In this isolation, the residue from an alcoholic sweet pea extract was partitioned between ether and water. The aqueous phase was purified by treatment with basic lead acetate followed by passage through the strongly basic anion-exchange Amberlite IRA-400 (hydroxyl form). The toxic factor was then adsorbed on the strongly acidic cation-exchange resin Amberlite IR-105 (H) (*3*), from which it was eluted with 5 percent H_2SO_4. Excess H_2SO_4 was removed with barium hydroxide. After concentration of the solution to a small volume, the addition of alcohol caused the precipitation of an unidentified alkali sulfate, which was removed by filtering the warm solution. Fine, white needles separated on cooling. These sintered and decomposed at about 225°C and consisted of the sulfate of an amine. When they were fed to rats, they gave rise to the typical skeletal changes seen in odoratism (sweet pea lathyrism).

More recently, active crystals have been isolated from sweet peas by means of the procedure used in the following isolation.

Coarsely ground sweet peas (1400 g) were placed in a large Soxhlet extractor and were thoroughly extracted with *n* hexane (Skelly Solve B) to remove lipids. The peas were then dried and extracted continuously with 95 percent ethyl alcohol for 7 hr. The alcoholic extract was allowed to cool and to stand overnight. During this time, dense rosettes of yellowish crystals formed in the extract. These crystals, which represented the impure toxic factor, were purified as follows.

The supernatant solution was decanted, and the crystals were washed several times with ethyl ether to remove small amounts of oily material. The crystals were dissolved in 90 ml of water and were treated with a solution of basic lead acetate until no more precipitation occurred. Following filtration, excess lead was removed with hydrogen sulfide, and the lead sulfide filtrate was evaporated to dryness under reduced pressure. The white, solid residue was dissolved in boiling alcohol with the gradual addition of small amounts of water. On cooling, crystals formed which were filtered with suction, washed with 95 percent alcohol, and dried. The yield was 1.6 g; mp 197°C with decomposition.

Recrystallization from alcohol-water mixtures gave very fine, long, colorless needles melting at 209° to 210°C, uncor. (Fig. 1). The nitrogen content by semimicro-Kjeldahl determination was 21.3 percent (21.25 percent; 21.29 percent) (*4*).

When fed to rats at a level of 0.2 percent of the diet, the crystals were somewhat less effective in producing skeletal changes in the animals than were sweet pea diets containing 25 percent sweet peas.

The crystalline material has the following properties: it is very soluble in water, is insoluble in ether, and has limited solubility in alcohol. It gives a positive ninhydrin reaction. During the isolation studies, it was determined that the toxic factor dialyzes readily through Visking cellulose and that it is not precipi-

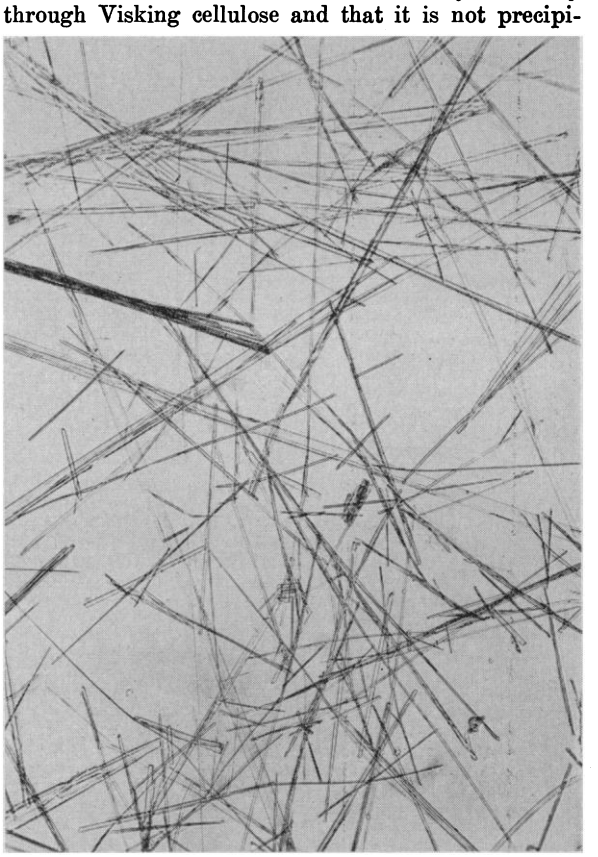

Fig. 1. Toxic crystals isolated from sweet peas (×100).

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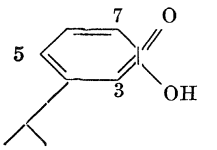
Hinokitiol, isolated from the phenolic part of the essential oil of *Chamaecyparis obtusa* Zieb et Zucc., is 4-isopropyltropolone (*1*). From this structure, it is



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.