0.5 hr. of the test, and with the shortest interval between sessions. These conclusions are based on the higher correlations between the two tests for the first 0.5 hr. and for the 1-day interval. The disadvantage of the greater decrement in activity after the shortest intersession interval is offset by the generally higher correlations between the first and second sessions found for the 1-day group, as well as by the practical advantage of using a shorter interval. These findings may be of use to experimenters in selecting the optimum conditions for testing the effect of chlorpromazine on the activity of mice in the photocell cage.

REFERENCES

(1) Furgiuele, A. R., Kinnard, W. J., and Buckley, J. P., *J. Pharm. Sci.*, **50**, 252(1961).
(2) Watzman, N., Barry, H., III, Kinnard, W. J., and Buckley, J. P., *Federation Proc.*, **23**, 197(1964).
(3) Weaver, J. E., and Miya, T. S., *J. Pharm. Sci.*, **50**, 910 (1961).
(4) Irwin, S., *Rev. Can. Biol.*, **20**, 239(1961).
(5) Winer, B. J., "Statistical Principles in Experimental Design," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 202.
(6) Cronbach, L. J., "Essentials of Psychological Testing," 2nd ed., Harper and Row, New York, N. Y., 1960, p. 141.
(7) Edwards, A. L., "Statistical Methods for the Behavioral Sciences," Holt, Rinehart, and Winston, New York, N. Y., 1954, p. 304.
(8) Adler, M. W., *Psychopharmacologia*, **5**, 393(1964).
(9) Rushton, R., Steinberg, H., and Tinson, C., *Brit. J. Pharmacol.*, **20**, 99(1963).

Preliminary Investigations of *Heracleum mantegazzianum*

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Preliminary investigations of the air-dried roots of *Heracleum mantegazzianum* grown under greenhouse conditions were conducted. Seed germination requires moist cold treatment. The germination rate appears to be directly related to the length of the moist cold period. Gibberellin seed treatment did not substitute for the cold requirement. Thin-layer chromatography revealed the presence of 6 coumarins which were tentatively identified as bergapten, isobergapten, pimpinellen, isopimpinellen, sphondin, and umbelliferone. Results also indicate that the plant can cause photosensitization.

EXCELLENT reviews concerning the distribution, chemistry, or pharmacological properties of the naturally occurring coumarins are available (1–5); included are phytochemical studies revealing the presence of coumarins in a number of *Heracleum* species. A notable aspect is the involvement of furocoumarins in certain cases of phytophotodermatitis; several of them occur in *Heracleum* species. The distribution of photodynamically active furocoumarins were recently reviewed by Pathak *et al.* (6). Although phytochemical investigations of the coumarins of *Heracleum* species has been extensive, the species *Heracleum mantegazzianum* Somm. et Lev. is a noteworthy exception. However, during the course of this investigation, a report by Beyrich (7) revealed the presence of phellopterin and other coumarins in this species, but the results are not entirely consistent with those reported in this investigation. Since *H. mantegazzianum* has been reported to evoke phytodermatitis, a preliminary investigation was undertaken to determine the presence of photosensitizing coumarins and related compounds.

To obtain sufficient plant material for this and subsequent studies the plant was propagated under greenhouse conditions. Seeds of the *Umbelliferae* have been noted for germination difficulties, and germination standards for cultivated members of this order have been set much lower than those of other plants (8). The seeds of some *Heracleum* species have a requirement for after ripening in moist cold (9). Attempts have also been made to obviate the cold requirement in the dormancy of certain seeds by chemical means, especially with the gibberellins (10). Since no report could be found in the literature concerning the cold requirement of the effects of gibberellic acid on seed germination of *H. mantegazzianum*, preliminary germination studies were also conducted.

EXPERIMENTAL AND RESULTS

Germination Studies.—Preliminary studies were designed to compare the effect of cold treatment *versus* treatment with gibberellic acid on the germination rate of the seeds. Three groups of seeds (39 per group) were planted in flats containing a mixture of 1 part sand and 2 parts sandy loam plus 50 Gm. of complete fertilizer.[1] Group *A* was pretreated by storage at 2–5° for 74 days; group *B* was soaked for 20 hr. in a solution of gibberellic acid (100 p.p.m.); and group *C*, soaked in distilled water, was considered the control group. The flats were maintained under normal greenhouse conditions and germination was allowed to occur at a temperature range of 18–27° for 38 days. Ger-

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[1] Organic Morcrop, Chas. Lilly Co., Seattle, Wash. (Analysis: 5% total nitrogen, 3% available phosphate, 2% available potash.)

mination did not occur in group *B* or group *C;* however, 4 seeds in group *A* germinated. Although this is a low germination rate (10.3%), it was apparent that cold treatment is required for germination. Based on these results, 1000 selected seeds were soaked for 36 hr. and refrigerated at 2–5° for 294 days. This treatment resulted in a 55% germination.

Preparation of Root Material.—To obtain root material for subsequent investigations, seeds were cold treated and germinated on moist blotting paper in Petri dishes. After germination occurred, they were transplanted in peat pots containing the soil mixture previously described. After 92 days the plants (average height 33.5 cm.) were harvested, the roots washed free of soil, and separated from the plant. The roots were allowed to dry at room temperature for 8 weeks. The dried material was milled to a No. 20 powder in a Wiley mill and the powder placed in air-tight colored glass containers until utilized in subsequent analyses.

Extraction of Coumarins.—Approximately 13.5 Gm. of dried powdered root was placed in a Soxhlet-type apparatus and extracted to exhaustion with 100 ml. of ethyl ether. The extractive was filtered and the filtrate evaporated on a steam bath to a syrupy residue. The residue was washed 3 times with 2-ml. portions of petroleum ether and redissolved in a mixture containing equal parts of ethyl ether and alcohol to a total volume of 10 ml. in a volumetric flask. The extract was stored in a refrigerator until subjected to chromatographic analysis.

Chromatographic Analysis.—Tentative identification of the coumarins was accomplished by employing two-dimensional thin-layer chromatography on Silica Gel G. The extract was applied in 20-μl. portions to thin-layer plates, 200 mm. on each side. The plates were developed in ethyl acetate–xylene (1:1) in the first direction and hexane–ethyl acetate (2:1) in the second direction. The solvent front was allowed to proceed a distance of 100 mm. in each direction. Solutions of known coumarins which were chromatographed singly, in mixture, and in combination with extracts, were compared to chromatoplates of extracts without additions. The coumarins were detected by examining the plates under ultraviolet light. This procedure revealed the presence of 7 principal spots which were tentatively identified as bergapten, isobergapten, pimpinellin, isopimpinellin, sphondin, and umbelliferone. The seventh spot, which fluoresced blue, was not identified, but co-chromatography indicated that it was not imperatorin.

DISCUSSION

The germination studies indicated that seeds of *H. mantegazzianum* will germinate only after extended cold treatment since no seeds germinated in the group where this procedure was omitted. Seeds exposed to moist cold for 74 days showed a germination rate of 10.3%. When the cold period was extended to 294 days, the germination rate increased to 55%. It appears, therefore, that the germination rate may be directly related to the period of moist cold treatment. An attempt to overcome this requirement by soaking in a solution of gibberellic acid met with no success. Similar attempts by Stuart and Cathey (10), but with sweet cherry and peach, indicated that gibberellic acid only partially substituted for cold treatment. Although gibberellins have been credited with substituting for other germination conditions, *i.e.*, light requirement, they do not adequately substitute for the cold treatment (10).

Thin-layer chromatographic analysis of the ether extract of air-dried root revealed the probable presence of 6 coumarins: bergapten, isobergapten, pimpinellin, isopimpinellin, sphondin, and umbelliferone, the last being the only nonfurocoumarin. A seventh spot, which fluoresced blue, was not imperatorin. A preliminary report by Beyrich (7), who investigated the fruits of *H. mantegazzianum*, showed the presence of pimpinellin and isopimpinellin in addition to the following furocoumarins: angelicin, xanthotoxin, imperatorin, and phellopterin. Since he examined a different plant part, direct comparison of results is precluded.

The plant under investigation has been shown to evoke phytophotodermatitis (6). The furocoumarins, especially bergapten and xanthotoxin, cause dermatitis. Since these compounds in all probability appear to exist in *H. mantegazzianum*, as well as others possibly having similar biological activity, this plant should be considered a photosensitizing species.

REFERENCES

(1) Karrer, W., "Konstitution und Vorkommen der Organischen Pflazenstoffe," Birkenhauser Verlag, Basel und Stuttgart, 1958, p. 531.
(2) Späth, E., *Ber.*, **70A**, 83(1937).
(3) Reppel, L., *Pharmazie*, **9**, 278(1954).
(4) Soine, T. O., *J. Pharm. Sci.*, **53**, 231(1964).
(5) Bose, P. K., *J. Indian Chem. Soc.*, **35**, 367(1958).
(6) Pathak, M. A., *et al.*, *J. Invest. Dermatol.*, **39**, 225 (1962).
(7) Beyrich, T., *Naturwiss.*, **52**, 133(1965).
(8) Robinson, R. W., *Botan. Rev.*, **20**, 531(1954).
(9) Stokes, P., *Ann. Botany*, **16**, 441(1952).
(10) Stuart, N. W., and Cathey, H. M., *Ann. Rev. Plant Physiol.*, **12**, 369(1961).