

rubber bung at right-angles to its surface. By standing the cylinder in hot water and filling the borer with hot water, air bubbles in the wax are avoided (Fig. 2). (3) Substitution of cold for hot water sets the wax. First immerse the cylinder in cold water then empty the cork borer and refill it with cold water. (4) When the wax has hardened, hot water inside the borer enables it to be withdrawn after rapid removal of the bung at the first sensation of softening of the wax in contact with it. The surface of the wax remains smooth and the angles clean cut. (5) A tightly fitting bung firmly pressed into place minimizes loss past the coverslip. A very light smearing of the sides of the bung with silicone oil (as used in the flotation process) can be used to prevent this loss, which has proved minimal in our experience.

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D. L. GLEN

University Department of Surgery,
Western Infirmary,
Glasgow.

¹ Alexander, R. F., and Spriggs, A. I., *J. Clin. Path.*, **13**, 414 (1960).

² Long, L., Roberts, S., McGrath, R., and McGrew, E., *J. Amer. Med. Assoc.*, **170**, 1785 (1959).

³ Morley, T. P., *Canad. J. Surg.*, **2**, 363 (1959).

⁴ Roberts, S., Watne, A., McGrath, R., McGrew, E., and Cole, W. H., *Amer. Med. Assoc. Arch. Surg.*, **76**, 334 (1958).

⁵ Sandberg, A. A., and Moore, G. E., *J. Nat. Cancer Inst.*, **19**, 1 (1957).

⁶ Engell, H. C., *Acta Chirurgica Scand.*, Supp. 201, Chap. 2 (1955).

⁷ Fisher, E. R., and Turnbull, R. B., *Surg. Gynec. Obstet.*, **100**, 102 (1955).

⁸ Poole, E. H., and Dunlop, G. R., *Amer. J. Cancer*, **21**, 99 (1934).

⁹ Kuper, S. W. A., Bignall, J. R., and Luckock, E. D., *Lancet*, **i**, 852 (1961).

¹⁰ Malmgren, R. A., Pruitt, J. C., del Vecchio, P. R., and Potter, J. F., *J. Nat. Cancer Inst.*, **20**, 1203 (1958).

¹¹ Seal, S. H., *Cancer*, **12**, 590 (1959).

Growth-promoting Activity of Caffeic Acid

Vendrig and Buffel have recently stated¹ that trans-caffeic acid "may be a very important natural growth regulator, not less important than indole-3-acetic acid". This conclusion was based on the identification of caffeic acid as one of the active growth substances in an ether extract of leaves, and on growth tests with commercial caffeic acid on 2-mm. sections of *Avena* coleoptiles. We believe that the true explanation of these observations is quite different. In what follows, a good crystalline sample of commercial caffeic acid, m.p. 188°–190°, was used.

In the first place, if caffeic acid were a true auxin it should produce inward curvatures in the slit pea stem

Table 1. GROWTH ACTIVITY IN PEA CURVATURE TEST IN DEGREES*

	Concentration - moles/l.†				
	10 ⁻³	10 ⁻⁴	10 ⁻⁵	3 × 10 ⁻⁶	0
Indole-3-acetic acid	—	+314	+140	+16	-115
Caffeic acid	-34	-108	-170	—	-149

*Outward curvature shown as minus, inward as plus. Angle of curvature measured between tangent to the tip of the slit section and projection of unslit section of stem. Duration of test, 24 hr. at 25°, following 1 hr. in distilled water. Another test following 4 hr. in distilled water gave similar results.

† All solutions except water control were brought to pH 5.5 with 0.1 N sodium hydroxide.

Table 2. GROWTH OF *Avena* COLEOPTILE SECTIONS IN SOLUTIONS OF CAFFEIC ACID AND OTHER POLYPHENOLS WITH AND WITHOUT INDOLE-3-ACETIC ACID

Solution		Without	Plus
		indole-3-acetic acid	indole-3-acetic acid 25 µgm./l.
Water		22.5	22.8
Caffeic acid	3 × 10 ⁻⁶ M	20.3	24.8
Caffeic acid	6 × 10 ⁻⁶ M	26.9	29.0
Caffeic acid	1 × 10 ⁻⁵ M	19.9	26.9
Chlorogenic acid	1 × 10 ⁻⁵ M	24.4	33.6
Catechol	1 × 10 ⁻⁵ M	19.9	31.1
Hydroquinone	1 × 10 ⁻⁵ M	21.5	33.1
Indole-3-acetic acid	500 µgm./l.	39.8	
Indole-3-acetic acid	1,000 µgm./l.	46.4	

Growth expressed as percentage of the initial length; sections 3 mm. long; time, 24 hr.

test, for this test is less specific than that with *Avena* coleoptiles². Table 1 shows, however, that if it has any real activity this does not exceed 0.1 per cent of that of indole-3-acetic acid. It is suggested that the apparent activity of caffeic acid in the reported *Avena* test is due to synergism with traces of indole-3-acetic acid.

One of us has shown³ that caffeic acid and other diphenolic substances produce marked synergistic effects with indole-3-acetic acid. A test carried out in the exact manner described by Vendrig and Buffel¹ is shown in Table 2. *Avena* coleoptile sections 3 mm. long were floated for 6 hr. on water, then on the test solutions and measured after 24 hr., using a photographic enlarger. Since no sucrose was added, the growth increments were smaller than usually reported from this laboratory. It will be seen, however, that caffeic acid alone, at the same concentrations as those reported, shows little real growth activity under these conditions. However, in presence of a level of indole-3-acetic acid which by itself has no effect, caffeic acid produces a small but significant growth increment, about 10–25 per cent beyond that of the indole-3-acetic acid control. Chlorogenic acid⁴, catechol and hydroquinone have similar effects. These actions probably rest on the inhibition of the indole-3-acetic acid oxidizing system⁵.

Aqueous solutions of caffeic acid contain an equilibrium mixture of the *cis*- and *trans*-forms⁶, of which the *cis*-form could perhaps comprise as much as 10 per cent. It is thus not excluded that the very low level of true auxin activity shown by caffeic acid may be due to the *cis*-component. An activity comparable to that of *cis*-cinnamic acid, which averages 2 per cent of that of indole-3-acetic acid, could be compatible with the present data. What is known of the effect of OH groups on the relation between structure and auxin activity⁷ would make any higher value most improbable.

Our results, therefore, lend no support to the suggestion that caffeic acid has any important level of true auxin activity, and indicate that the observed effects are due to synergism with endogenous indole-3-acetic acid.

K. V. THIMANN
M. TOMASZEWSKI
W. L. PORTER

Harvard Biological Laboratories,
Cambridge 38, Mass.

¹ Vendrig, J. C., and Buffel, K., *Nature*, **192**, 276 (1961).

² Thimann, K. V., in *Fundamental Aspects of Normal and Malignant Growth*, edit. by Nowinski, W. W., 757 (Elsevier Pub. Co., Amsterdam, 1960).

³ Tomaszewski, M., *Abstr. Fifth Intern. Cong. Biochem.*, Moscow, **15**, 94, 1404 (1961).

⁴ Tomaszewski, M., *Bull. l'Acad. Polon. Sci. Classe II*, **7**, 127 (1959).

⁵ Kenten, R. H., *Biochem. J.*, **59**, 119 (1955). Cf. Ray, P. M., *Ann. Rev. Plant Physiol.*, **9**, 81 (1958).

⁶ Williams, A. H., *Chem. and Indust.*, 120 (1955).

⁷ See, for example, Thimann, K. V., *Plant Physiol.*, **33**, 311 (1958).