SHORT COMMUNICATIONS

IDENTIFICATION OF CHERRY AND HAGEMAN'S "COMPOUND XI" FROM MAIZE AS CHELIDONIC ACID*

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During examination of the nucleotide fraction of barley seedlings a compound that resembled an unidentified constituent of maize shoots—"compound XI" (Cherry and Hageman 1960; Cherry *et al.* 1961)—was found. The materials from barley and from maize have now been identified as chelidonic acid (4-oxopyran-2,6-dicarboxylic acid); this compound is present in a number of species of grasses (Table 1). In tissues and in extracts at pH values above 2 this strong acid exists mainly in an anionic form (cf. Miyamoto and Brochmann-Hanssen 1962) and is referred to below as chelidonate.

Materials and Methods

Barley, maize, oats, and wheat (for details of species and varieties see Table 1) were grown in the dark at 25°C on screens over 0·2 mM $CaSO_4$ after sterilization with hypochlorite. Shoots and roots were collected when the former were 4–8 cm long.

The aerial parts of pasture grasses growing after spring rains were kindly provided by Mr. J. H. Silsbury.

Chelidonate was extracted from plant tissue (1–2 g) by blending for 1 min at maximum speed in a homogenizer (Bühler, Tübingen, Germany) with 50 ml of ethanol–acetic acid–water (200 : 1 : 199 by vol.). The suspension was centrifuged for 15 min at 2000 *g*. The supernatant fraction was filtered (Whatman No. 541 paper) and 30 ml of filtrate was passed through two 45-mm disks of anion-exchange paper (Whatman DE 20), without suction, in a filtration apparatus (Millipore Filter Corp., Bedford, Mass., U.S.A.). The disks were washed with 100 ml water and chelidonic acid (together with other strong acids) was eluted with 4N formic acid (5 × 2 ml); the residue after removal of solvent at 40°C/15 mmHg was subjected to electrophoresis (Markham 1955) on Whatman No. 40 paper in 0·05M citrate (Tris, pH 4·2) at 26 V/cm for 1 hr. Chelidonate bands from the plant extract and from an authentic sample (anodic migration *c.* 20 cm) were located by contact printing on reflex document paper with a low-pressure mercury lamp and were eluted with 7 ml water at 95–100°C. Spectra of the extracts and of blanks were recorded with a Unicam SP.700 spectrophotometer; cells of 2 cm light path were used and the concentration of chelidonate was calculated from extinction values at 270 mμ and 313 mμ ($\epsilon_{270m\mu} - \epsilon_{313m\mu} = 10 \cdot 3 \times 10^3$ in the conditions of this assay; final pH 4·5–5·5).

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Compound XI from maize was purified by gradient elution from Dowex-1 formate as described by Cherry and Hageman (1960); these authors had reported its high electrophoretic mobility and its absorption maximum ($\lambda_{max.}$) at 270 mμ (see Results and Discussion section) but did not characterize the compound further.

Results and Discussion

The acid from barley and compound XI from maize resembled chelidonic acid in the following respects: absorption maxima at 267 and 273 mμ, minima at 243 and 270 mμ, and an inflexion at 285 mμ at pH 5–7 (with lower resolution the double

TABLE 1

CHELIDONATE IN SOME SPECIES OF GRAMINEAE

Analysis was by the spectrophotometric method described in the Materials and Methods section

Species and Variety of Plant	Parts Analysed	Concentration of Chelidonate (μmoles/g fresh wt.)
Avena sativa L., cv. Victory (oats)	Shoots	0·16
	Roots	0·005
Dactylis glomerata L. (cocksfoot)	Aerial parts	0·10
Ehrharta calycina Sm. (veldt grass)	Aerial parts	0·11
Hordeum vulgare L., cv. Prior A (barley)	Shoots	0·29
	Roots	0·01
Lolium perenne L. (perennial ryegrass)	Aerial parts	0·02
L. rigidum Gaud. (Wimmera ryegrass)	Aerial parts	0·13
Phalaris tuberosa L.	Aerial parts	0·7
Triticum durum Desf., cv. Dural (wheat)	Shoots	<0·005
	Roots	<0·005
Zea mays L., cv. Golden Cross Bantam (maize)	Shoots	0·86
	Primary roots	0·10
	Secondary roots	0·03

maxima merge as a single maximum at 270 mμ; cf. Attenburrow *et al.* 1945); absorption maximum at 270 mμ and minimum at 243 mμ in 0·1N H_2SO_4; R_F 0·07 in butan-1-ol–acetic acid–water (20 : 3 : 7 by vol.); R_F 0·15 in isobutyric acid–aq. 0·42N NH_3 (33 : 17 v/v); R_F 0·31 in ammonium sulphate–0·1M sodium phosphate (pH 6·8)–propan-1-ol (30 : 50 : 1 w/v/v). Synthetic chelidonate and the material from plants examined here could not be separated by electrophoresis in 0·05M citrate (Tris, pH 4·2), mobility 0·79 $cm^2\ V^{-1}\ hr^{-1}$, or in 0·05M borate (Na^+, pH 9·1), mobility 1·02 $cm^2\ V^{-1}\ hr^{-1}$. Chelidonic acid was eluted from Dowex-1 at the same point in the formic acid–ammonium formate gradient (Cherry and Hageman 1960) as compound XI from maize. From these results it is concluded that the material isolated from the species of Gramineae listed in Table 1 is chelidonic acid although large enough quantities for microanalysis were not obtained.

Since Probst's (1839) discovery of chelidonate in *Chelidonium majus* the compound has been found in at least 340 plant species (15 families) but no report of its occurrence in the Gramineae has been found (cf. Buch 1960). In the most extensive

investigations (Ramstad 1953; Kwasniewski 1953) colour reactions and microscopic tests were used. The combination of anion exchange, electrophoresis, and spectrophotometry used in the present investigation is more convenient for quantitative analysis of chelidonate. The compound was found in all the species of Gramineae examined (Table 1) except *Triticum durum* Desf. cv. Dural; shoots and roots from this variety contained traces of material that absorbed light at 270 mμ and migrated at the same rate as chelidonate on electrophoresis but the level was below the limit for reliable detection of chelidonate by this method (0·005 μmole/g fresh wt.).

Chelidonate is known to inhibit plant growth at concentrations as low as 10 μM; in the presence of indolylacetate it resembles coumarin in stimulating pea seedlings and *Avena* coleoptiles at this concentration (Leopold *et al.* 1952). The concentration of chelidonate in the *Avena* coleoptiles examined in the present investigation was more than 100 μM (Table 1) and the presence of this active material in a system that is widely used for studies of stimulation or inhibition of growth indicates a need for caution in the interpretation of results from such studies. Cherry and Hageman (1960) had reported that compound XI (133 μg/ml, i.e. 0·7 mM chelidonate) inhibited tissue respiration and growth of root tip sections. This concentration was found in maize shoots, but roots contained much less chelidonate (Table 1). The distribution of chelidonate between vacuoles and other regions of plant tissues is not known, and it cannot be concluded that the effects of endogenous chelidonate resemble those found when the material is added to these systems.

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