Phytochemistry, Vol. 20, No. 5, pp. 1133–1134, 1981.
Printed in Great Britain.

0031–9422/81/051133–02 $02.00/0
Pergamon Press Ltd.

CAFFEYL ESTERS OF GLUCARIC ACID IN *LYCOPERSICON ESCULENTUM* LEAVES

C. A. ELLIGER, R. E. LUNDIN and W. F. HADDON

Western Regional Research Center, USDA, SEA, AR, Berkeley, CA 94710, U.S.A.

(*Received* 7 *August* 1980)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; caffeic acid; aldaric acids; insect growth inhibitors; *Heliothis zea*; tomato fruitworm; host plant resistance.

Abstract—Caffeic acid esters of a mixture of glucaric acid and lactone forms occur in tomato leaves (*Lycopersicon esculentum*). Hydrolysis of these materials yields only caffeic acid and glucaric acid. The esters described are inhibitory to the growth and development of the tomato fruitworm (*Heliothis zea*) and represent one of the several resistance factors of the plant.

In a survey of tomato for substances inhibiting the larvae of the tomato fruitworm (*Heliothis zea* Boddie) [1] we have observed several significant inhibitory chemical factors. These include the steroidal glycoalkaloid, α-tomatine; the flavonol diglycoside, rutin; the widely encountered depside, chlorogenic acid; and a complex mixture of caffeyl glucaric acid and lactone forms which are described in this communication. Although glucaric acid occurs in a variety of plants [2, 3], and hydroxycinnamates are very frequently found in conjugated form [4, 5], this is the first observed instance of esterification of caffeic acid to a hexaric acid moiety.

Tomato leaf material was extracted sequentially with Me_2CO, MeOH and H_2O. The MeOH extract contained most of the α-tomatine as well as the rutin and chlorogenic acid. The H_2O extract was passed through XAD-2 (Mallinckrodt) which served to remove residual rutin and chlorogenic acid and gave an effluent which still contained phenolic substances and which suppressed the growth of *H. zea* in our bioassay procedure [6]. Following concentration of this aqueous solution and chromatography upon Sephadex G-25, *ca* 1% of a mixture of phenolic acids was obtained (based on original dry wt) which possessed all the antigrowth activity of the H_2O extract. Preliminary examination of UV and ^1H NMR spectra of the total mixture indicated that these materials were caffeic acid esters of a highly polar carbohydrate. We also observed that the components were more highly retained on Sephadex G-25 at pH 3 than at pH 7 which is consistent with the presence of a free carboxyl group, and which, indeed, paralleled the behavior of chlorogenic acid in the same system. Repeated chromatography on Sephadex G-25 and LH-20 gave two nearly homogeneous acids as major components of the mixture as well as impure samples of related materials. Progress of the separations was conveniently monitored by HPLC of column fractions using an Ultrasphere Octyl (Altex/Beckman) column and a solvent system of H_2O–MeOH (4:1) containing 0.5% formic acid.

From the syrupy water solutions of the purer samples were obtained small amounts of crystalline solids, designated **1** and **2** in order of elution from G-25. Both compounds showed infrared bands associated with a carboxylic acid group, but **2** gave a much more complex set of maxima in the carbonyl region which could be ascribed to the presence of a lactone ring. That both **1** and **2** possessed the caffeyl ester structure was evident from the ^1H NMR and UV spectra, the latter confirming the presence of a free *o*-dihydroxyphenyl functionality (borate shift) [7]. Mass spectral information obtained on the per-TMSi ether/ester derivatives of **1** and **2** indicated that **1** was a caffeyl tetrahydroxyhexanedioic acid (m/z = 876, hepta-TMSi) and that **2** was a lactone (m/z = 714, penta-TMSi). High resolution mass measurements upon each M^+ yielded empirical formulae in agreement with open and lactone forms for **1** and **2**, respectively.

Hydrolysis of both **1** and **2** gave caffeic acid, identified by comparison with authentic material, and glucaric acid admixed with lactone forms. A portion of the latter substance was converted to the disodium salt and thence to its TMSi derivative which avoided the complication of lactonization [8]. GC comparison on several stationary phases using the TMSi derivative of authentic glucaric acid bore out the identity of the isolated acid [8, 9]. Additionally, reduction of the aldaric acid to the hexitol and formation of the alditol acetate [8] gave material whose ^1H NMR and GC properties were identical with those of sorbitol hexa-acetate, thus confirming identification of the original substance as glucaric acid. In a similar manner, the entire crude mixture of acid/esters obtained from tomato leaf was subjected to hydrolysis. Only caffeic and glucaric acids were produced. We conclude, therefore, that the insect growth inhibiting substances present in the water extract of tomato leaves are mixed caffeyl glucaric acid and lactone forms. We have not, however, addressed ourselves to the problem of point of attachment of the caffeyl group or to the various lactone possibilities.

EXPERIMENTAL

Anhydrous leaf material of *L. esculentum* Mill. (cv Campbell-29), 240 g, was ground in succession with 3 × 2 l. Me_2CO, 1.5 l.

MeOH and 2 l. H_2O using a Waring blender at high speed for 2 min. The H_2O extract obtained on centrifugation was stirred with 500 ml of XAD-2 resin for 2.5 hr and filtered. After concn to *ca* 150 ml *in vacuo*, the resulting soln was chromatographed in 3 batches on a 450 × 75 mm Sephadex G-25 (medium) column with 0.5% HOAc in H_2O. The zone of elution vol. 2.5–4 l. contained active material, 2.5 g. Further chromatography of this mixture on a 50 × 950 mm Sephadex G-25 (superfine) column yielded **1** (elution vol. 2900–3250 ml) and **2** (elution vol. 3300–3650 ml). Additional purification of **2** was accomplished by chromatography on Sephadex LH-20 with MeOH–H_2O (9:1) containing 0.5% HOAc (elution vol. 2375–2750 ml). From H_2O solns were deposited crystals of **1**, mp > 300°, dec. at 200°, and **2** mp 194–195°.

Spectra. **1** showed IR absorption (ν_{max}^{KBr}) in the 2400–3600 cm^{-1} region consistent with a strongly hydrogen-bonded OH as well as a broad carbonyl band centered at 1710 cm^{-1}. UV λ_{max}^{MeOH} nm: 330, 302, 244 and 235; λ_{max}^{MeOH} nm (borate): 350, 304 and 255 nm. The ^1H NMR spectrum of **1** consisted of a poorly resolved set of multiplets between 3.8 and 5.4 ppm as well as characteristic signals associated with the caffeyl group: 7.67 and 6.32 (*d*, J = 16 Hz, 1 H each, olefinic protons) and 7.08 (*d*, J = 2 Hz, 1 H), 6.96 (*d*, *d*, J_o = 9 Hz, J_m = 2 Hz, 1 H) and 6.77 (*d*, J = 9 Hz, 1 H) aromatic protons.

Component **2** differed significantly only in the IR spectrum which showed lactone absorption at 1740 in addition to a distinguishable shoulder at 1720 and a well-defined band at 1690 cm^{-1} corresponding to carboxylic acid and conjugated ester respectively (ν_{max}^{KBr}).

Pertrimethylsilylation of **1** and **2** was effected with Tri-Sil (Pierce Chemical), and MS determination obtained thereon: **1** $(TMSi_7)$, m/z = 876.3344. $C_{36}H_{72}O_{11}Si_7$ requires 876.3459. **2** $(TMSi)_5$, m/z = 714.2581. $C_{30}H_{54}O_{10}Si_5$ requires 714.2563.

Hydrolyses. In a 50 ml flask equipped with a N_2 inlet were placed 100 mg of ester and 10 ml H_2O. After warming and purging to displace O_2, 100 mg of NaOH was then added, and the mixture was stirred 2.5 hr at room temp. Excess HCl was then added (pH *ca* 2), and caffeic acid separated from soln. After filtration of the solid, the remaining caffeic acid was recovered from the filtrate by adsorption onto 10 ml of XAD-2 resin followed by subsequent desorption with MeOH. Material isolated was identical in all respects to commercial caffeic acid. The soln of glucaric acid was taken to dryness, and the solid triturated with MeOH. Filtration removed most NaCl, and the glucaric acid was used without further purification.

Reduction. *Ca* 50 mg of glucaric acid was stirred overnight with 25 ml of MeOH and 1 g of anhydrous Dowex-50 followed by filtration and evaporation of the resulting ester/lactone mixture. The product obtained was taken up in 25 ml of THF and 100 mg of $LiAlH_4$ was added, and the suspension stirred 2 hr at room temp. Excess H_2O was added and solvent removed *in vacuo*. Acetylation was carried out at 80° using Ac_2O and KOAc. The resulting impure sorbitol hexaacetate was purified by chromatography on a Whatman Partisil M-9 10/50 column using $CHCl_3$ containing 0.75% EtOH. The acetate obtained from the naturally occurring substances was identical to commercial sorbitol hexaacetate spectroscopically and by GC on OV-225 at 250°.

Preparation of TMSi *glucaric derivatives.* The glucaric acid/lactone mixture (5 mg) resulting from hydrolysis was adjusted to pH 10 with dil NaOH and taken to dryness under N_2. To the glucarate salt was added 0.5 ml of Tri-Sil, and the mixture heated at 80° for 1 hr. GC determinations were carried out using the following stationary phases: OV-1, QF-1 and OV-17. In all cases R_fs were identical to those of authentic glucaric per-TMSi.

REFERENCES

1. Elliger, C. A., Wong, Y., Chan, B. G. and Waiss, A. C., Jr. (1981) *J. Chem. Ecol.* (accepted.)
2. Winsnes, R. and Andrew, M. (1970) *Acta Chem. Scand.* **24**, 3428.
3. Kringstad, R. and Nordal, A. (1975) *Phytochemistry* **14**, 1868.
4. Robinson, T. (1963) *The Organic Constituents of Higher Plants*, pp. 48–50. Burgess, Minneapolis.
5. Zenk, M. H. (1979) in *Recent Advances in Phytochemistry* (Swain, T., Harborne, J. and Van Sumere, C. F., eds.) Vol. 12, pp. 139–176. Plenum Press, New York.
6. Chan, B. G., Waiss, A. C., Jr., Stanley, W. L. and Goodban, A. E. (1978) *J. Econ. Entomol.* **71**, 366.
7. Jurd, L. (1956) *Arch. Biochem. Biophys.* **66**, 284.
8. Kringstad, R. and Bakke, I. L. F. (1977) *J. Chromatogr.* **144**, 209.
9. Jansen, L. and Samuelson, O. (1971) *J. Chromatogr.* **57**, 353.