3-O-CAFFEOYLSHIKIMIC ACID (DACTYLIFRIC ACID) AND ITS ISOMERS, A NEW CLASS OF ENZYMIC BROWNING SUBSTRATES[1]

V. P. Maier, D. M. Metzler and A. F. Huber

U. S. Department of Agriculture
Fruit and Vegetable Chemistry Laboratory[2]
263 S. Chester Avenue
Pasadena, California

Received October 22, 1963

Previously, chromatographic and UV spectral evidence of several hydroxycinnamoylshikimic acids has been reported, but their structures were not elucidated (Goldschmid and Hergert, 1961; Hanson and Zucker, 1963). During studies on enzymic browning substrates in dates (Phoenix dactylifera) we isolated a new crystalline compound, which we named dactylifric acid (Maier and Metzler, 1963). It is one of the main enzymic browning substrates in dates. We have now established its structure to be 3-O-caffeoylshikimic acid by degradation and synthesis. This is the first isolation and synthesis of a crystalline hydroxycinnamoylshikimic acid.

Dactylifric acid was extracted with methanol from fresh green dates and purified by column chromatography on silicic acid. After recrystallization from water it gave m.p. 224-225° (decomp.); C 57.50, H 4.80%, calcd. for $C_{16}H_{16}O_8$: C 57.16, H 4.79%; $[\alpha]_D^{25}$ -124° (c=1.16 in 62.5% aqueous ethanol) and neutral equivalent 345, calcd. 336.

---

[1] Presented in part at the 144th American Chemical Society Meeting, Los Angeles California, April, 1963; work supported in part by Date Administrative Committee, Indio, California.

[2] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service.

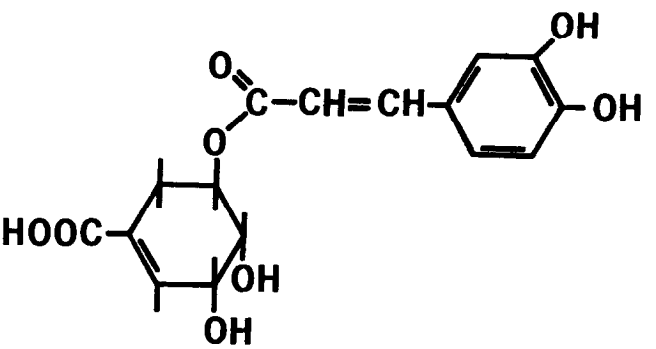
The UV spectra of the compound with various reagents were as follows: EtOH λ_{max} 332, ~303 mμ; EtOH + NaOAc λ_{max} 331, ~304 mμ; EtOH + NaOAc + H_3BO_3 λ_{max} 349, ~306 mμ. These spectra show the presence of a caffeic acid moiety in which the o-dihydroxy group is free whereas the carboxyl group is blocked (Jurd, 1956; 1957). Enzymic hydrolysis of the compound with anthocyanase B (Corner et al., 1962) yielded caffeic and shikimic acids. Shikimic acid was also obtained on both acid and base hydrolysis. Caffeic and shikimic acids were identified by comparison with authentic samples using paper chromatography and electrophoresis, silicic acid chromatostrips, and color reactions. Caffeic acid was also confirmed by UV and IR spectra and mixed melting point.

Hydrogenation of 5 mg of dactylifric acid in the presence of 10 mg of 10% Pd-C catalyst in methanol used 2.33 moles of hydrogen per mole (assuming a molecular weight of 336). Under the same conditions, caffeic acid absorbed 1.01 moles/mole as expected and shikimic acid absorbed 1.39 moles/mole. The hydrogen uptake of shikimic acid in excess of one mole appears to be due to hydrogenolysis of the allylic hydroxyl group. Thus, the 2.33 moles/mole of hydrogen taken up by dactylifric acid agrees with that found for the sum of caffeic and shikimic acids separately, i. e., 2.40 moles/mole.

These data establish that dactylifric acid is a monocaffeoylshikimic acid.

Treatment of several milligrams of dactylifric acid with HCl in dry acetone to test for the formation of an isopropylidene derivative gave an amorphous white solid in high yield. On paper chromatograms and electrophoretograms the compound gave a single spot which had a different R_f value than dactylifric acid. Since the UV spectrum of the isopropylidene derivative was essentially identical to that of dactylifric acid the caffeic acid portion of the molecule was unchanged. Similar treatment of shikimic acid is known to yield

only 4, 5-O-isopropylidene shikimic acid (Fischer and Dangschat, 1937), since the 3-hydroxyl is trans. Enzymic hydrolysis of isopropylidene dactylifric acid yielded caffeic acid and 4, 5-O-isopropylidene shikimic acid, as identified by chromatographic comparison with authentic samples. The formation of a 4, 5-O-isopropylidene derivative of the shikimic acid portion of dactylifric acid shows that dactylifric acid is 3-O-caffeoylshikimic acid:



Dactylifric Acid, 3-O-Caffeoylshikimic Acid

The structure of dactylifric acid was confirmed by synthesis. Diphenylmethylenedioxycaffeoyl chloride was condensed with 4, 5-O-isopropylidene shikimic acid (Fischer and Dangschat, 1937). Hydrolysis of the blocking groups with aqueous acetic acid - HCl yielded crystalline 3-O-caffeoylshikimic acid after chromatographic purification. This compound was shown to be identical with dactylifric acid by chromatography, electrophoresis, UV and IR spectra and mixed melting point. In addition, condensation of 3, 4-dimethoxycinnamoyl chloride with methyl (4, 5-O-isopropylidene) shikimate followed by hydrolysis of the isopropylidene group with aqueous acetic acid gave methyl 3-O-(di-O-methyl-caffeoyl) shikimate. This compound was chromatographically and electrophoretically identical with dactylifric acid methylated with diazomethane.

A second crystalline material which elutes from the column more slowly than dactylifric acid was also isolated. It yields only caffeic and shikimic acids on hydrolysis, has a UV spectrum identical with dactylifric acid, a neutral equivalent of 341 and absorbs 2.26 moles of hydrogen/mole. When the material is tested on an analytical silicic acid partitioning column (Hanson, 1963) a major and minor band are observed with R_{caf}[4] values of 2.38 and 2.12, respectively. Dactylifric acid has an R_{caf} value of 1.83. Both bands have UV spectra identical with that of dactylifric acid and similar R_f values in 10% acetic acid and 0.1N HCl. These compounds are therefore isomers of dactylifric acid and the names isodactylifric acid and neodactylifric acid are suggested for the major and minor components, respectively. After methylation of the phenolic hydroxyls and the carboxyl with diazomethane isodactylifric acid gave a negative periodate test (Metzenberg and Mitchell, 1954) whereas neodactylifric acid gave a positive test. Thus, it is tentatively concluded that isodactylifric acid is 4-O-caffeoylshikimic acid and that neodactylifric acid is 5-O-caffeoylshikimic acid.

When paper chromatograms of pure dactylifric acid and its isomers are sprayed with either a crude enzyme extract of dates or with mushroom tyrosinase brown colors develop in every case. Dactylifric and isodactylifric acids were also treated with the date extract and tyrosinase in acetate buffer, pH 5.1. Each gave a yellow-brown color after 10 minutes at room temperature. These tests establish the dactylifric acids as new enzymic browning substrates.

The isolation and structural determination of a crystalline caffeoylshikimic acid now firmly establishes the existence of a new group of substituted cinnamic acid esters based on shikimic acid. It is possible that the substituted cinnamic acid esters of shikimic acid will be difficult to detect in plants because they may not accumulate to any appreciable extent due to high

[4]Ratio of effluent volume of the compound to that of caffeic acid.

metabolic activity. The importance of shikimic acid in the biosynthesis of aromatic compounds such as tyrosine, phenylalanine, the substituted cinnamic acids, flavonoids, coumarins and liguin (Brown, 1961) suggests this possibility. Whether compounds of the caffeoylshikimic acid type are directly involved in or associated with any of these biosynthetic pathways remains to be determined.

ACKNOWLEDGEMENTS

We would like to thank Drs. Robert M. Horowitz and David L. Dreyer for their many helpful suggestions.

REFERENCES

Brown, S. A. Science 134, 305 (1961).
Corner, J. J., Harbourne, J. B., Humphries, S. G., and Ollis, W. D. Phytochem. 1, 73 (1962).
Fischer, H. O. L. and Dangschat, G. Helv. Chim. Acta 20, 705 (1937).
Goldschmid, O. and Hergert, H. L. Tappi 44, 858 (1961).
Hanson, R. H. and Zucker, M. J. Biol. Chem. 238, 1105 (1963).
Jurd, L. Arch. Biochem. Biophys. 63, 376 (1956).
Jurd, L. Arch. Biochem. Biophys. 66, 284 (1957).
Maier, V. P. and Metzler, D. M. Amer. Chem. Soc. Abst. 144th Meeting, IC (1963).
Metzenberg, R. L. and Mitchell, H. K. J. Amer. Chem. Soc. 76, 4187 (1954).